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Control of Northern Fowl Mite in Community Wire Cages with Malathion in Special Dust-Bath-Boxes[1]

J. L. Rodriguez, Jr., and L. A. Riehl, *University of California Citrus Experiment Station, Riverside*

Abstract

The northern fowl mite, *Ornithonyssus sylviarum* (C. & F.), on hens in community wire cages was controlled effectively by putting a dust-bath-box (18×12×6 inches deep) charged with 5 pounds of 4% malathion dust in a cage for 14 days. The hens were free of mites for 6 weeks thereafter. In comparison the control program of the ranch consisted of a general spray application each 30 days. The results suggested adaptation of the method to a program of spot-treating only the cages where mites were found on the eggs. Data on the percentages of cages requiring spot-treatment each week for a period of 47 weeks are discussed. The evidence offers several reasons to recommend spot-treatment with dust-bath-boxes charged with 4% malathion dust as a method of choice for controlling the northern fowl mite on chickens in community wire cages. Additional trials conducted to determine dosage efficiency showed that 1 pound of 4% malathion dust per box per 30 hens was optimum for mite control. In these trials reducing the depth of the box to 3 inches improved its acceptance by the hens and eliminated any problem of eggs laid in the dust-bath-box.

In southern California many commercial poultry ranches house their laying flocks in community-type wire mesh cages (Rodriguez & Riehl 1958). Here the northern fowl mite, *Ornithonyssus sylviarum* (C. & F.), can appear at any time of the year. Control measures are used generally. Malathion is a pesticide of choice; its high efficiency against the northern fowl mite was demonstrated by Vincent *et al.* (1954). However, infestations recurred at too short an interval even when the treatments were made carefully and thoroughly.

Early detection of this mite requires the constant vigilance of the personnel gathering the eggs, and this appears to be one of the most important factors of control. Although some operators spray the entire ranch when a few infested pens are found, most wait until the mites become heavy in a number of pens. Also, treating on a predetermined schedule, with intervals of 30 days even if no mites are seen, is a common practice.

Mass spraying with high pressure equipment was observed by the authors on two large poultry ranches. At the approach of the spray rig the hens were scared; they fluttered about and on to each other in piles in the farthest corner of the cage. Good spray coverage of all birds in a cage is hard to obtain under such conditions; there is a good likelihood that a few birds will be missed completely and others dampened only indirectly while many of the hens are drenched. In addition, the disturbance and scaring accompanying spraying is a very serious emotional stress to the flock. Individual hens caught in the piles may be bruised or more severely injured. The inherent nature of the highly excitable high-producing laying hen is not amendable to such harsh treatment and laying may be interrupted.

Problems of coverage and scaring are important to dusting as well as spraying. While the birds are not wet inhalation of dust particles with consequent irritation to the respiratory system becomes a factor.

The inherent difficulties involved in mass spraying or dusting emphasized the need to investigate other means of applying insecticides to the birds. Rodriguez & Riehl (1957) demonstrated the effective control of body lice of hens on litter with malathion in dust-bath-boxes. This method offered interesting possibilities for use in community wire cages. Self-application of dust as a means of pest control is natural for the hens.

Results of experiments to investigate the dust-bath-box charged with malathion dust for control of the northern fowl mite on laying hens in community wire cages are reported here.

Materials and Methods.—Studies were conducted during 1957 and 1958 on a large poultry ranch near Anaheim. Each community wire cage contained 70 to 100 adult white Leghorn hens. Cages infested with the northern fowl mite occurred throughout a block of 360 cages.

In the first experiment wooden boxes 18 inches long by 12 inches wide and 12 inches deep were constructed of 1 by 6 inch No. 3 pine lumber. One box was used in each cage and only the actual weighed amount of malathion dust was placed in the box. The length of the box was governed by the size of the door to the cage. The depth was the same as that of the boxes used in earlier experiments (Rodriguez & Riehl 1957). The boxes 12 inches deep were not accepted rapidly by many of the hens and

[1] Paper No. 1205, University of California Citrus Experiment Station, Riverside. Accepted for publication February 23, 1959.

The pesticide chemicals discussed may not be used unless a tolerance has been established or an exemption from the requirement of a tolerance has been granted for each specific use, irrespective of the information contained in this report.

good control of the northern fowl mite was obtained only when relatively long intervals were allowed for acceptance and treatment.

Observations of the actions of the chickens suggested that hens in community wire cages might prefer a shallower box. Therefore, the boxes were modified by reducing the depth from 12 to 6 inches. When boxes 6 inches deep were put in the cages, the hens accepted them at once.

An experiment was started with the special boxes; one dust-bath-box 18 inches long by 12 inches wide by 6 inches deep was used per cage. The boxes were charged with 5 pounds of either 4% or 10% malathion dust before they were put in a cage. Each treatment was replicated in 12 cages while a single cage was used as the untreated check. The presence of mites on the eggs indicated that each of the pens selected for the experiment was heavily infested with the northern fowl mite. In each of the cages chosen, five hens taken at random per cage were examined individually each counting date; if mites were found, the number present were counted. The dust-bath-boxes were left in the cages for 14 days and then removed. By the 14th day the charge of 5 pounds of malathion dust had been completely used in most of the cages. Differences in the rate of use were noted between pens. In some, the charge of dust was gone by the seventh day and no mites were found in the counts. Although this was noted in the observations the boxes were left in these pens until the 14th day.

DISCUSSION OF RESULTS.—Nine of the 12 cages treated with 4% dust were free of mites in 7 days; in the other three cages a few hens were found with mites, but the intensity of the infestation had been reduced from very heavy to light (10 to 20) or very light (1 to 4) mites per hen. The overall average of infested hens for the 12 pens was 14%. By 14 days all the hens were clean and remained free of mites for 6 weeks thereafter. The grower wished to cull and replace hens 56 days after the experiment started so observations were discontinued. At 56 days, 3 to 4 mites per bird were found on hens in 4 of the 12 cages treated with 4% malathion dust, an overall average of 16% hens infested; cages treated with 10% malathion dust did not have mites.

During the period of 2 weeks allowed for the hens to accept the box in the preliminary experiments with boxes 12 inches deep, an average of 2.40 eggs per box were found for the period. Records of the ranch showed an average of 0.18 eggs on the open floor per day per cage, or 2.52 eggs in 14 days. Thus, in the daily routine it is necessary to inspect the interior of each cage for eggs on the open floor as well as to remove dead, sick, or cull birds. Removing any eggs laid in the dust-bath-box during an interval of 2 weeks would not require additional time or effort. Choice as a site for laying eggs is deterred when the box is being actively used as a dust-bath. After the first few days and particularly when the charge of dust is gone some of the hens will begin to lay eggs in the box. It would be practical to take the boxes out of the cages as soon as the dust is gone.

Observations made by the authors indicate that as an infestation of northern fowl mite starts, it is characteristic for the mites to become apparent in scattered separated cages, *i.e.*, spot locations, representing an involvement of only a fraction of the total cages. Studies by Rodriguez & Riehl (1959) with hens in individual cages showed that the overall control for the period of a year was better with a program of spot-treatments with 4% malathion dust as needed than with complete applications to all cages and that insecticide and labor for the spot-treatments were considerably less for the year than for a single application to all cages.

Table 1.—Number and percentage of community cages spot-treated per month with 5 pounds of 4% malathion dust per cage applied in a special dust-bath-box[a] placed in the cage and left there for an interval of 7 days only during a program of spot-treatments at two poultry ranches to control the northern fowl mite and the total number of treated cages requiring retreatment within periods of 30 and 120 days after the initial treatment.

	Total Number Cages Spot-Treated				Total Number Cages Retreated			
	Ranch A		Ranch B		Within 30 Days		Within 120 Days	
Month	No.	Per Cent	No.	Per Cent	Ranch A	Ranch B	Ranch A	Ranch B
March	67	18.6	—[b]	—	1	—[b]	0	—[b]
April	77	21.4	—	—	1	—	6	—
May	23	6.4	36	6.9	1	0	6	0
June	6	1.7	46	8.8	0	1	0	3
July	0	0.0	9	1.7	0	1	0	2
Aug.	1	0.3	43	8.3	0	0	0	16
Sept.	0	0.0	43	8.3	0	0	0	4
Oct.	1	0.3	21	4.0	0	0	0	0
Nov.	18	5.0	44	7.9	0	1	0	3
Dec.	32	8.9	68	12.2	1	5	2	5
Jan.	48	13.3	50	8.9	2	1	6	3

[a] Dimensions 18 inches long by 18 inches wide by 6 inches deep.
[b] Spot-treatment program started at Ranch B during the week ending May 3, 1958.

Using dust-bath-boxes in community wire cages lends itself readily to a program of spot-treatments. Infested cages can be detected by looking for mites on the eggs each day as they are gathered. Trials were started in a block of 360 cages (Ranch A) by the operator of the ranch used for the above experiments. The ranch personnel looked for mites on eggs, put the boxes in the infested cages, and kept a record of the cages treated. The dimensions of the box were 18 inches long by 18 inches wide by 6 inches deep; a charge of 5 pounds of 4% malathion dust was put in the box before it was placed in the cage. For purposes of the work program, the decision was made to leave the box in the cage for 7 days and then remove it. The record of cages treated was kept for each week.[2] These data are summarized in table 1 by months since in general the nature of the control is indicated as well by the total for each month as for each week.

The control program used previously on this ranch consisted of 0.5% malathion in aqueous spray (prepared with a liquid emulsifiable concentrate formulation) applied to all of the cages, with a power sprayer, each 30 days, or occasionally each 15 days as necessary.

During the first and second months respectively (table 1) 18.6% and 21.4% of the cages of Ranch A were spot-treated; the cost of malathion was approximately the same for the spot-treatment vs. spray programs. However, during the third and fourth months there was a considerable reduction in the number of cages requiring treatment and the comparable costs of malathion for the

[2] Through the courtesy of Mr. E. B. Clements, 15381 Anaheim Road, Anaheim, California.

spot-treatments decreased to approximately $\frac{1}{3}$ and $\frac{1}{16}$ of that of spraying with applications at intervals of 30 days. On the other hand the important costs of emotional disturbance of the hens and of application labor were considerably less for the spot-treatment program; for it, the work can be done as the eggs are gathered rather than the special allocation of the time of several people required for spraying. Stress to the hens from northern fowl mites would be alleviated better by spot-treatments with dust-bath-boxes than by spraying since treatment is made in a cage in the incipient stage of the mite infestation and distribution of an adequate amount of insecticide to each hen is accomplished more effectively.

During the first week of May 1958 spot-treatments with the special dust-bath-box were started at a second ranch (B) with 520 community cages; results are reported in table 1. Although reinfestation from northern fowl mite infestations in the poultry ranches bordering it was considerably greater at Ranch B than at Ranch A, the spot-treatment program controlled the mite in more than 90% of the cages. The spot-treatment procedure was executed better at Ranch A with better vigilance in looking for mites when the eggs were gathered and closer follow-up with a spot-treatment. Although vigilant detection and immediate treatment are critical to the success of the control, they are not unreasonable requirements.

On the other hand, the overall control of northern fowl mite at Ranch B was better with the spot-treatment than with the general spray program used previously, and the cost of malathion for the spot-treatments was less than 40% of the cost of spraying.

The data shown in table 1 show that some of the cages had to be retreated; this may be expected since in some pens the hens will not be as active in using the dust-bath-box as in others. The evidence of the preliminary trial indicated that the mites will be controlled if the box is left in the cage until the malathion dust is gone. The operator of the ranch used in the experiments thinks it is preferable to take the boxes out on a definite schedule with a time interval based on the rate of use in most of the cages (*i.e.*, 7 days) and to retreat cages as necessary. This detail is optional. Most of the intervals between treatment of the retreated cages were relatively long. In these the likelihood may be as good that the reinfestation came from a source outside the cage as that it was a carry-over of the previous infestation.

When the evidence showed that the dust-bath-box was an effective method of application against northern fowl mite on hens in community cages, several trials were conducted in an experiment to determine the amount of dust actually required per box per unit of hens for efficient control. Reducing the depth of the box from 6 inches to 3 inches was tried also; therefore, the dimensions of the boxes used in the trials were 18 inches long by 12 inches wide by 3 inches deep. The depth of 3 inches appears preferable because no eggs were laid in the boxes 3 inches deep even when they were left in the cage as long as 30 days. The range of 12 to 18 inches seems to be acceptable for the width.

Trials were conducted in four types of community cages varying in size, shape and number of hens, the Jackson-type with floor dimensions 10 feet by 8 feet and 90 hens per cage, the Munson-type (5′ by 4′) with 15 hens per cage, and two sizes of the Grow-lay-type, an 8′ by 4′ with 28 hens and a 4′ by 2′ with 7 hens per cage. In practice the amount of dust used was varied from 1 to 5 pounds per box per unit of 90 hens in the Jackson-type community cage; the equivalent of 3 pounds per box was used in each of the Munson and the Grow-lay types. For this paper the amounts of dust will be given in pounds per unit of 30 birds. The charge of dust was put in the box when it was put in the cage; most of the hens accepted the boxes immediately. Treatments were replicated, varying from 11 to 20 times.

The activity of the hens, rather than the amount of dust, appeared to govern the rate of use. The box was left in the cage until the charge of dust was gone; this varied from 7 to 14 days in most cages to 30 days in a few cages.

On each counting date a sample of hens taken at random was examined in each cage; the sample consisted of 10 hens in the Jackson-type cages and of five hens in each

Table 2.—Average percentages of hens infested with northern fowl mites in several types of community cages at intervals after a dust-bath-box[a] containing various charges (pounds per 30 hens), respectively, of 4% malathion dust was put in the cages.

		Jackson-Type (10′ by 8′)					Munson Type (5′ by 4′)	Grow-Lay-Type (8′ by 4′)	Grow-Lay-Type (4′ by 2′)
Days After Treatment	Check	Pounds of Dust per Box per 30 Hens							
		1.66	1.33	1.00	0.66	0.33	1.00	1.00	1.00
		Average Percentages of Hens Infested							
Before Treatment	100	100	100	100	100	100	100	100	100
7	100	0	0	0	9.6	14.1	0	0	0
14	100	0	0	0	6.9	17.0	0	0	0
28	100	0	0	0	10.9	27.2	0	0	0
56	100	0	0	0	32.7	32.0	0	0	0
84	100	0	0	0	50.0	35.6	0	0	0
100	100	0	8.6	5.3	50.0	55.6	—[b]	—[b]	—[b]
120	100	9.0	10.0	10.5	66.7	62.5	—	—	—

[a] Dimensions 18 inches long by 12 inches wide by 3 inches deep.
[b] Counts discontinued because hens were culled.

of the Munson and Grow-lay types. The hen was considered infested if any mites were found.

The average percentages of hens infested with northern fowl mites following treatment with various amounts (pounds per unit of 30 hens) of 4% malathion dust applied in dust-bath-boxes are given in table 2. The evidence indicates that 1 pound of 4% malathion dust per dust-bath-box per unit of approximately 30 hens is optimum for efficient control of northern fowl mite on hens in community cages; complete control was obtained within a week after application and maintained for 84 days. The zero level of hens infested was not attained with either the 2 or 1 pound dosages per box per 90 hens.

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Biology of the Brown Wheat Mite[1]

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ABSTRACT

In greenhouse studies brown wheat mites (*Petrobia latens* (Müll.)) were confined individually in cages suspended on small grain plants. The average incubation period of active eggs ranged from 8.5 to 11.2 days. The time from hatching to adult required 9.8 to 11.0 days, two-thirds of this time being spent in quiescence between instars. The egg incubation period was more dependent on temperature than the larval and nymphal periods. Adult life ranged from 21.5 days in 1955 to 10.6 days in 1957. Egg production also varied greatly from year to year. Diapause eggs required moisture for hatching, but excessive amounts were detrimental to the other stages.

In field studies in Utah and Idaho diapause eggs were found to be the source of overwintered populations as well as populations that developed on grain crops in the fall. Mite populations on the plants varied greatly throughout a day, being greatest in the morning and evening when temperatures were between 70° and 80° F.

The first described specimens of the brown wheat mite (*Petrobia latens* (Müll.)) in the United States were collected in California and New Mexico in 1911 (Banks 1912). Ten years later this mite was found causing severe injury to wheat in Idaho (Ewing 1922). Since that time it has been reported periodically as a pest of agriculture (Jones & Mecom 1935, Fenton & Whitehead 1944, Knowlton & Ma 1950, Reynolds & Swift 1951, Knowlton & Tibbetts 1952). Henderson & Tilton (1955) and Pritchard & Baker (1955) regarded this mite as an important pest of agriculture in the southwestern and western United States.

In spite of the apparent importance of this mite, there is little information on its life history, damage, and control. Pritchard (1953) suggested a need for research to resolve apparent conflicts in the literature with regard to its biology. For example, Fenton (1951) regarded the mite as strictly a dry-weather pest, but Baker & Pritchard (1953) reported that it flourished during winter rains in the western United States. To obtain more information on its biology, greenhouse and field studies were conducted in Utah and Idaho from 1955 to 1958.

GREENHOUSE STUDIES.—*Materials and Methods.*—Mites were confined in small cages attached to the leaves of small-grain plants. The cages (fig. 1) were modifications of those described by Kaloostian (1955), being made of dialyzing cellophane paper, casein glue, and steel washers. Magnets were placed on the underside of the leaves to hold the cages on the upper side. Double-bar magnets were required on plants with prominent midribs.

Head-band binoculars and a hand lens were of use throughout the experiments, and frequently a binocular dissecting microscope was necessary. At first a sable-hair brush was used to transfer newly hatched mites to the cages, but later a small aspirator was designed to fit into the base of the cage. Transfers from cage to cage subsequent to the larval stage were made by placing the two cages base to base with the one containing a mite on top; a sharp tap caused the mite to drop into the lower cage.

One larva that had hatched during the previous 24-hour period was confined in each cage, and daily thereafter the stage of development was recorded. All manipulations and observations of mites were made during the same time interval each day.

In 1955–56 several varieties of both wheat and barley were the host plants. In 1957 Velvon barley was the host, and in 1957–58 Atlas barley. The plants were grown in either clay or disposable fiber pots or planted directly in the floor soil of the greenhouse.

A hygrothermograph was in operation during all experiments.

Pre-adult Stages.—As indicated in table 1, active eggs required an incubation period of 8.5 to 11.2 days, depending on the temperature. In 1957 when the period was shorter than in the other 2 years, eggs hatched in 10.8 days during January and February when the average temperature was 71° F., and in 7.7 days during March and April when it was 80°. Fenton (1951) reported an incubation period of 6 to 7 days at 75° and 52% rela-

[1] Accepted for publication February 26, 1960.

[2] In cooperation with the Utah Agricultural Experiment Station, Logan, Utah. Stanford McClellan, Fred Legner, and Neil Ballif assisted in these studies.

tive humidity. Although the incubation period varied a great deal, the variation was not confined to eggs laid by different adults. For example, the incubation periods of eggs laid by three adults on the same day were 6 to 12 days, 7 to 24 days, and 8 to 28 days. Eggs with a greatly prolonged incubation period are, from the practical point of view, nonviable.

The brown wheat mite has one larval and two nymphal instars with a period of quiescence between each instar. The mite molts at the termination of each quiescent period. The time spent in each instar or period of quiescence varied only a few hours from year to year.

The total time from larva to adult ranged from 9.8 to 11.0 days. Fenton (1951) reported 8 to 9 days. Apparently temperature had less effect on this period than on embryonic development. This phenomenon would aid in propagation of the species, for adverse weather would retard hatching but would not deter mite development once an egg had hatched. Although the average greenhouse temperatures were similar to those under field conditions, field temperatures reach greater upper extremes, especially in the soil where eggs incubate and larvae and nymphs are quiescent.

Adult Stage.—Adults that emerged from the quiescent deutonymph stage entered a preoviposition period which lasted 1.6 to 1.7 days. Fenton (1951) reported a similar period of 1 to 2 days. Frequently there was a postoviposition period of 1 day before the mite died. The mite was moribund during this latter period.

Adults lived for an average of 21.5 days in 1955–56, 10.6 days in 1957, and 12.8 days in 1957–58. Fenton (1951) reported an adult life of 3 weeks. There are three possible reasons for the differences between the 3 years: 1) There may have been varietal or genetic differences owing to the collection of eggs from different geographic locations. The eggs used in 1955–56 were collected in southwestern Salt Lake Valley, Utah, those utilized in 1957 in Pocatello Valley, Box Elder County, Utah, and eggs for the 1957–58 studies came from Downey, Bannock County, Idaho. 2) The 1955–56 studies were conducted at lower temperatures than the other years. 3) The 1955–56 studies were conducted under conditions of higher relative humidity.

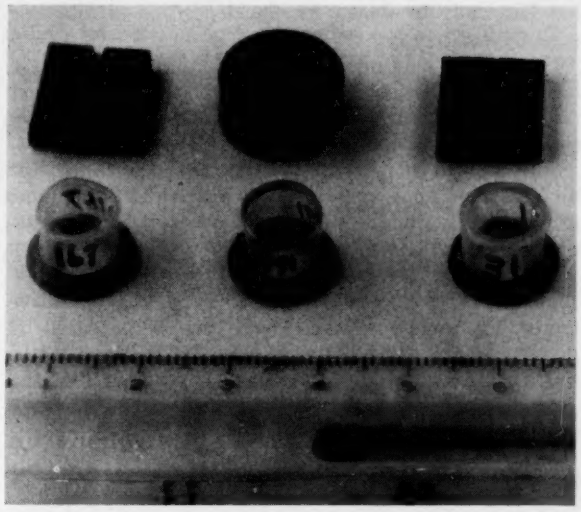

FIG. 1.—Three sizes of cell cages and two types of magnets used to hold them on the leaves of small plants. The bar magnets held the cages flat against leaves with prominent midribs.

The mites under observation in 1955–56 deposited more active eggs than those in the later 2 years. During 1957–58 only about half as many eggs were deposited as during the first year. The one adult observed laying diapause eggs in 1955 deposited 34. Although the averages in 1957 and 1958 were far less, they were based on more observations.

Lees (1953a, b) reported that in *Panonychus ulmi* (Koch) the initiation of diapause egg deposition was due to low temperatures, short photoperiods, or restricted food supply. Zein-Eldin (1956) concluded that none of these factors caused diapause eggs in *Petrobia apicalis* (Banks). He suggested that their age at the time of

Table 1.—Life history, longevity, and fecundity of brown wheat mites reared in the greenhouse.

Stage	1955–56 (68° F. and 49% RH)			1957 (76° F. and 28% RH)			1957–58 (74° F. and 31% RH)		
	Duration of Stage (Days)		No. of Mites Observed	Duration of Stage (Days)		No. of Mites Observed	Duration of Stage (Days)		No. of Mites Observed
	Range[a]	Average		Range[a]	Average		Range[a]	Average	
Active egg	8–22	11.2	370	6–28	8.5	821	6–93	11.2	2,276
Larva	0–1	0.7	78	0– 1	0.6	37	0– 2	0.7	159
First quiescence	1– 4	2.2	78	1– 5	2.7	37	1– 5	2.3	159
Protonymph	0– 3	1.2	78	0– 2	1.0	37	0– 4	1.4	159
Second quiescence	1– 3	1.8	78	1– 4	2.1	37	0– 4	2.1	159
Deutonymph	1– 4	1.4	78	0– 4	1.2	37	0– 6	1.7	159
Third quiescence	2– 5	2.5	78	0– 4	2.4	37	1– 5	2.8	159
Total pre-adult growth	8–13	9.8	78	8–12	10.0	37	7–17	11.0	159
Preoviposition period	0– 4	1.7	129	0– 3	1.6	37	0– 6	1.7	165
Generation (egg to egg)	16–39	22.7	78	15–45	20.1	37	13–116	23.9	159
Adult	1–39	21.5	40	3–19	10.6	37	2– 25	12.8	165
Egg deposition:	*Number per mite*			*Number per mite*			*Number per mite*		
Active	0–138	57	37	2–98	41	29	1–98	27	145
Diapause	—	34	1	3–32	16	8	1–22	8	20

[a] 0 indicates less than 24 hours.

hatching may be related to the deposition of diapause eggs by their progeny.

No experiments were conducted to evaluate these factors in relation to diapause eggs of the brown wheat mite, but observations indicated the causes to be rather complex and possibly to include these factors. For example, in 1955–56 it was found that in the greenhouse at temperatures approximating those in the field in June, when adults normally would lay diapause eggs, only active eggs were laid on wheat or barley until the fourth generation. By that time the plants had become mature. Plant conditions were assumed to be the causative factor, since diapause eggs would then be produced by mites of any generation, including a few first-generation mites hatching from diapause eggs. However, in 1957 and 1958 diapause eggs were first laid in the fifth generation during January, when the photoperiod was far shorter than in May, when they are normally laid in the field. Furthermore, the plants were young and succulent, although the greenhouse temperature was about the same as field temperatures during June when plants are rapidly maturing. A few individuals in the sixth and eighth generations laid diapause eggs, but it was not until the ninth generation that a large number laid diapause eggs. The ninth-generation mites that produced diapause eggs were all the progeny of females which had fed upon plants that had either headed or were in the boot stage. On the other hand, some ninth-generation mites that produced active eggs were the progeny of females which fed upon the same plants. The progeny of eighth-generation mites which had fed on young, succulent plants all laid active eggs.

Diapause Eggs and Moisture.—Zein-Eldin (1956) reported that summer eggs of *P. apicalis* have a diapause of 2 to 3 months, after which hatching can be induced by a combination of low temperature (58° to 60° F.) and high humidity (80% or more). Diapause, considered to be arrested embryonic development for purposes of this discussion, in summer eggs of the brown wheat mite lasts for an indefinite period during hot, dry weather, after which soil moisture will generally initiate hatching within a few days during periods of favorable warmth. A lowering of temperatures does not seem to be necessary. Supplying moisture at laboratory room temperature will usually bring about hatching within a few days if the eggs have spent enough time in the field to complete the diapause. If not, they cannot be induced to hatch by the mere addition of moisture. When eggs were brought to the laboratory after a field exposure of no more than 6 hours, ice supplied under high vacuum to obtain absolute moisture failed to bring about hatching. Supplying contact moisture within a few weeks after oviposition also failed to induce hatching. These trials indicated that moisture will not induce hatching until the diapause is terminated. This was true even when the protective receptacle was removed from the egg.

In 1956 soil containing diapause eggs was collected from a field in Box Elder County, Utah. The first sample was obtained on September 13, while the soil was still warm, prior to rain or snowfall. It was kept dry and stored in the greenhouse. A second sample was collected on November 27, after both rain and snowfall, the ground being partially frozen. It was stored in an unheated garage. From each sample 200 eggs were obtained by loosening them from the soil with a dissecting needle under a stereomicroscope. On February 4, 1957, lots of 40 were placed on blotting paper in petri dishes. The eggs were surrounded by a barrier of petroleum jelly, and exposed to moisture by saturating the blotting paper for various periods. Exposure was terminated by rapidly drying the blotting paper. The eggs were then incubated at 72° F.

Table 2.—Effect of field moisture on the hatching of diapause eggs of the brown wheat mite.[a]

Hours of Contact with Saturated Blotting Paper	Eggs Collected from:	
	Dry Soil	Moist Soil
0	0	18
24	—[b]	26
48	20	32
72	35	30
96	33	34

[a] 40 eggs in each lot.
[b] Eggs killed by contact with petroleum jelly barrier.

As shown in table 2, eggs collected from warm, dry field soil did not hatch without exposure to moisture in the greenhouse, whereas 45% of those that had been exposed to field moisture did hatch without any additional. Maximum hatching of eggs from dry soil occurred after 72 hours. Since these eggs had not been exposed to cold weather and were kept in a warm environment, it appears that low temperatures were not important in promoting hatching. Maximum hatching of eggs that had been in cold, moist soil came after 48 hours. From 15% to 18% of the eggs of each sample did not hatch even after 96 hours. These eggs may not have been viable, or they may not have completed diapause.

FIELD STUDIES.—*Methods.*—Soil samples 4 to 5 inches deep were taken with a soil auger 5 inches in diameter and examined for eggs according to technique reported by Henderson (1960). Mite populations were obtained by means of the sampling-fork and greased-slide technique described by Henderson (1956).

Source of Infestation.—Only the diapause egg is important in the overwintering of the brown wheat mite in the Rocky Mountain region. Although larvae, nymphs, and adults were found during the fall of the year, live individuals were not present in the spring until the overwintered diapause eggs began to hatch. Viability of overwintered active eggs was extremely poor.

The diapause egg is also the source of fall infestation of grain crops. The dry, hot period between ripening of one crop and the fall planting of the next was found to be unfavorable to all other stages. Most active eggs hatched within 8 to 11 days after deposition and consequently could not contribute to the fall population. The almost complete absence of green vegetation in grain fields during the summer precluded a source of food for their development. The few immature mites that were observed on volunteer grain appeared to succumb to the extreme heat and dryness of the fallowed field.

Population trends of the brown wheat mite can be measured most accurately in the egg stage. Soil samples were taken periodically from two locations, the Salt Lake Valley near Bingham, Utah, and the Little Pocatello Valley in Box Elder County, Utah.

In the Salt Lake Valley in 1955 the diapause-egg population following the end of a fallow period reached a low of about 300 eggs per 5-inch core by the end of the year (table 3). The steady decline between late August and late November was caused by hatching. (The 570 found the following March 5 were probably due to sampling error. Thereafter, favorable climatic conditions allowed a heavy buildup during the spring of 1956, *e.g.*, on May 23 there were about 4,000 mites per foot of drill row. By June 11 there was a peak of nearly 9,300 diapause eggs per 5-inch core.

The data for Box Elder County (table 4) indicate that mite populations may reach economic proportions even when there were fewer diapause eggs. For example, early in May 1955 there were about 30 diapause eggs per core, but subsequent heavy rains probably killed and buried many eggs and newly hatched mites. In spite of these conditions, a very large mite population developed. More than 10,000 diapause eggs per core had been deposited by July 27. Although the diapause-egg population in the spring of 1957 was considerably larger than that in 1955,

Table 3.—Numbers of eggs and larvae of the brown wheat mite recovered in 5-inch cores of soil, Salt Lake Valley, Utah.

Date Sampled	Eggs		Larvae[a]
	Diapause	Active	
1955		*Field fallow*	
April 28	834	0	53
July 28	857	0	6
August 16	1,061	0	0
30	680	0	0
September 7		*Winter wheat planted*	
27	428	0	3
October 13	343	0	237
26	241	64	129
November 30	328	369	102
1956			
March 5	570	213	64
19	153	62	78
30	78	69	237
April 11	56	29	171
19	27	47	241
30	87	490	362
May 15	304	368	430
23	1,959	47	575
June 1	3,326	13	93
11	9,297	0	186
26		*Crop harvested*	
August 29	8,640	—	—
November 26	650	0	0
1957			
March 27	379	0	44
May 27	219	0	9
June 14	239	4	13
September 15 (ca.)		*Winter wheat planted*	
17	301	4	261
October 9	244	22	142
30	248	9	36
1958			
March 25	137	0	35
April 7	142	0	35
15	79	0	53
29	18	0	49
May 14	49	22	53

[a] Includes larvae in soil at time of sampling and larvae hatching from eggs during laboratory washing and handling of soil sample.

Table 4.—Egg populations of the brown wheat mite per 5-inch core of soil in spring barley and wheat, Box Elder County, Utah.

Date Sampled	Spring Barley		Wheat	
	Diapause	Active	Diapause	Active
1955				
May 7	*Crop planted*			
10	33	4	*Spring wheat planted*	
24	18	2	27	9
June 1–5	*Period of heavy rains, 2–3 inches*			
7	36	20	11	4
22	20	43	29	40
29	87	178	20	16
July 11	1,168	320	44	9
27	10,686	0	337	0
August 5 (ca.)	*Crops harvested*			
1955				
September 13	820	0	125	0
October 1 (ca.)			*Winter wheat planted*	
November 27	816	0	—	—
1957				
April 16	728	0	134	0
21 (ca.)	*Crop planted*			
May 11–29	*4.8 in. rain (several storms with over .5 in.)*			
May 27	159	18	—	—
June 14	18	182	71	555
August 5	*Crops harvested*			
20	9,235	0	—	—
October 29	3,736[a]	816	1,065	106

[a] Samples taken 400 feet north of previous sampling areas. Disking after August 20 resulted in inability to differentiate original areas.

severe rains again reduced the mite population. With the return of dry weather after May 29, the population increased until there were sufficient numbers to deposit more than 9,000 diapause eggs per core before soil samples were taken on August 20.

Abundance and Feeding Habits in Relation to Climate.—The apparent conflict between the reports of Fenton (1951) and Baker & Pritchard (1953) regarding the role of moisture in the development of brown wheat mite populations is easily resolved when the different climates are considered. The present authors found both wet and dry weather conducive to mite development. Although the hatching of diapause eggs requires moisture, subsequent growth is dependent upon a paucity of heavy rainfall. Therefore, in dry areas or seasons the rainy period may supply adequate moisture for hatching but not enough to deter mite growth. Heavy rains may destroy most of the mites. Rains up to $\frac{1}{4}$ inch had little destructive effect, but $\frac{1}{2}$ inch or more destroyed mites in any stage of development. Exceptions were fields that contained a large amount of small stones and gravel and were located on slopes, where even rains in excess of $\frac{1}{2}$ inch had little effect. It was postulated that mites are trapped in crevices and cracks by soil erosion during storms. In Utah and Idaho winter snows and ample spring rains provide moisture to initiate hatching of overwintered diapause eggs, while subsequent warmth and scarcity of rainfall allow the hatched mites to develop.

Mite populations in relation to soil surface and air temperatures were determined at 2-hour intervals from July 19 to 21, 1955, in Box Elder County. Soil surface temperature was determined with a mercury thermometer, the bulb of which was covered by a thin film of soil. Four

Table 5.—Abundance of the brown wheat mite on spring-planted wheat and barley in relation to temperature, Box Elder County, Utah, 1955.

Date and Time	Temperature (° F.)		No. of Mites per Foot of Row	
	Air	Soil	Barley	Wheat
July 19				
6 A.M.	57	59	2,164	928
8	77	76	2,910	946
10	88	100	1,760	330
12	93	128	1,198	270
2 P.M.	93	136	668	212
4	90	109	1,410	480
6	82	95	1,232	448
8	75	79	3,686	1,040
10	72	72	3,282	406
12	62	66	1,132	218
July 20				
2 A.M.	58	59	446	134
4	56	56	444	110
6	58	56	540	270
8	77	74	1,406	454
10	85	97	1,016	316
12	89	124	826	166
2 P.M.	90	122	774	180
4	94	110	1,246	324
6	88	92	2,046	490
8	75	78	2,332	618
10	70	70	1,202	262
12	67	66	1,004	146
July 21				
2 A.M.	62	63	516	68
4	63	61	188	26
6	62	64	452	192

Table 6.—Abundance of brown wheat mites on spring-planted barley in relation to climatic factors during a 48-hour period. Oneida County, Idaho, 1957.

Date and Time	Relative Humidity (%)	Temperature (° F.)			Wind (M.P.H.)	No. of Mites per Foot of Row
		Air	Soil Surface	Soil 1 in.		
July 8						
6 A.M.	55	55	54	—	2.2 NNW	420
8	45	71	79	—	2.0 S	2,407
10	28	81	105	—	1.5 SSE	2,317
12	19	83	115	—	5.0 S	1,584
2 P.M.	15	90	126	—	6.7 SSW	796
4	19	87	117	—	4.6 WSW	1,120
6	22	84	93	—	6.8 NW	2,064
8	29	75	75	—	0.1 NW	2,206
10	41	65	59	—	2.1 NNW	1,096
12	39	63	62	—	0	508
July 9						
2 A.M.	43	54	56	—	0.6 SW	295
3:30	46	56	59	—	0.9 NW	345
6	48	59	59	60	2.5 NNW	421
8	32	72	77	72	0	1,456
10	20	82	100	94	8.1 S	847
12	18	91	120	112	6.8 S	505
2 P.M.	16	92	147	113	7.1 S	405
4	14	92	118	106	4.2 SW	745
6	20	88	92	88	4.6 SW	1,047
8	28	75	72	74	0	1,894
10	53	67	66	68	1.1 NNW	1,803
12	42	54	59	64	0.9 NW	934
July 10						
2 A.M.	49	55	60	62	0.2 SE	565
4	54	54	60	61	0.9 NW	840

samples were taken at three locations in both a barley and a wheat field. The crops were in contiguous acreages that had both been planted to barley in 1953 and fallowed in 1954. Table 5 shows that mite abundance on the plants was correlated with temperature of both air and soil. The population reached a peak at both 8 A.M. and 8 P.M., when air and soil-surface temperatures were between 74° and 79° F. A statistical analysis of the data indicated that the optimum temperature was about 76° F.

On July 8 to 10, 1957, another population of mites was measured at 2-hour intervals in relation to various climatic factors in a field in northwestern Pocatello Valley, Oneida County, Idaho, that had been planted to spring barley in 1956. Four samples were taken at each of four locations in the field. During the second day the air and soil-surface temperature data were supplemented by soil temperatures taken at a depth of 1 inch. As shown in table 6, the number of mites appeared to be little affected by humidity or wind. On both July 8 and 9 the population was greatest at 8 A.M. and 8 P.M. At these times the air temperature was 71° to 75° F., and the soil-surface temperature 72° to 79° F.

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The Toxic Action of a Mixture of Sevin and *p,p'*-DDT to the House Fly[1]

SUMIO NAGASAWA[2]

ABSTRACT

The toxic action of a mixture of Sevin® (1-naphthyl methylcarbamate) and *p,p'*-DDT to adults of the house fly (*Musca domestica* L.) has shown a compound response curve which indicates that these two toxicants have different types of toxic action. Sevin was about one-ninth as toxic as *p,p'*-DDT.

A new carbamate insecticide, Sevin® (1-naphthyl methylcarbamate), which was introduced recently by the Union Carbide Chemical Company has great promise in the practical field because of its excellent toxicity to many agricultural pests and its low toxicity to mammals. The results of experiments carried out to determine the relative toxicities of Sevin and of *p,p'*-DDT to the house fly (*Musca domestica* L.) and the type of joint toxic action of a mixture of these two insecticides are reported in this paper.

MATERIALS AND METHODS.—The house fly used for the present work was the "Stauffer" strain separated in 1953 from the strain of the Stauffer Agricultural Research Laboratory at Mountain View, California. It has been reared in the insect toxicology laboratory of Berkeley at about 25° C. and a relative humidity of 60% to 70%, on a moist mixture of 1 part of rat and mice laboratory food and 3 parts of wheat bran for the rearing of larvae, and sugar and water for the adult flies.

Flies, 3 to 5 days old, were lightly anesthetized with carbon dioxide and sexed. Female flies, in groups of 30 to 40 individuals, were kept separately in 5-oz., kitchen-size, Dixie cups with sugar and water for 24 hours at laboratory condition (ca. 25° C.). Just prior to the application of insecticides, the flies were lightly anesthetized again with carbon dioxide. One mm.^{3} of an acetone solution of Sevin (m.p. 142° C.), or *p,p'*-DDT (m.p. 108° C.), or a 2 to 1 mixture of these two insecticides, was applied topically to the mesonotum using a micrometer-driven syringe. Acetone alone was applied as the control. After treatment the flies were transferred to a 150-cc. beaker with sugar and water, and were kept at 25° C. After 24 hours, the numbers of moribund and dead flies were recorded.

RESULTS AND DISCUSSION.—The results of the experiment, presented as the relation between dosage X (μg./fly) and mortality Y (%) for each insecticide and for the 2 to 1 mixture of these two insecticides, are shown in table 1. The mortalities shown in table 1 were corrected by Abbott's formula with the mortality in control (2.3%).

Calculation of Dosage-Mortality Curve.—Dosage X ($\times 100$) and mortality Y (%) shown in table 1 were transferred to logarithm x and probit y, respectively, and their relations were plotted on the graph. The calculation of the regression lines (ld–p line) and the χ^2-test for the agreement between the experimental data and the calculated results were made by the method described by Bliss (1935a, b). The results are shown in figure 1 and table 2.

Relative Toxicity of Sevin to p,p'-DDT.—If the regression lines for Sevin and *p,p'*-DDT shown in figure 1 were not parallel within the experimental error, in addition to the comparison of median lethal dose, it would be necessary to compare the toxicities at the point where the higher mortalities are obtained. However, the result of χ^2-test for the parallelism of two regression lines which was carried out by Bliss' method is $0.064 < 3.841$ ($n=1$), and it can be considered that these two regression lines are parallel. Therefore, it is enough to compare at the point of 50% mortality where the error is minimum. The LD_{50} values calculated are 3.102 μg./fly for Sevin and 0.357 μg./fly for *p,p'*-DDT. The relative toxicity of *p,p'*-DDT to Sevin is 8.69; that is, Sevin is about one-ninth as toxic as *p,p'*-DDT to female adults of the house fly. This ratio is in good agreement with the result of Eldefrawi *et al.* (1959), who found that Sevin was only about one-tenth as toxic as DDT to the house fly, the LD_{50} values, for topical application, being 0.2 μg./fly for DDT and 2.3 μg./fly for Sevin at the time of their tests.

Table 1.—Dosage *X*-mortality *Y* table for Sevin, *p,p'*-DDT, and their 2 to 1 combinations applied topically in acetone solution to female adults of *Musca domestica*.

Dosage (μg./Fly)	Sevin		*p,p'*-DDT		Mixture	
	No. of Insects	Mortality (%)	No. of Insects	Mortality (%)	No. of Insects	Mortality (%)
0.0625			114	13.8	127	1.6
0.125	110	2.3	107	35.9	110	6.0
0.25	115	5.6	116	40.8	117	18.6
0.5	119	18.3	114	55.1	157	36.0
1	120	30.1	120	65.9	117	50.2
2	113	42.9	109	86.9	108	82.9
4	109	61.5	126	91.1	120	93.1
8	123	63.4				

Joint Toxic Action of Mixture of Sevin and p,p'-DDT.—Bliss (1939) discussed the problem of predicting the toxicity of a mixture of poisons from knowledge of the effectiveness of the constituents separately, and distinguished three types of joint action, independent, similar, and synergistic with examples illustrative of each. His idea was developed by Finney (1942, 1947), Plackett & Hewlett (1948), and Hewlett & Plackett (1950) with the detailed mathematical representation. Following Finney (1947), the present type of joint toxic action of mixtures of 2 parts of Sevin and 1 part of *p,p'*-DDT shown in figure 1 seems to be an example of one of the compound types which may be explained with the following mathematical model.

When the two ld–p lines obtained by different insecticides are parallel within the experimental error and the mixture is applied as a total dosage, X, in which the proportion of the two insecticides are π_1, π_2, and if the probits, y, obtained for the mixture are satisfactorily represented by the following equation,

$$y = a_1 + b \log (\pi_1 + \rho_2 \pi_2) X,$$

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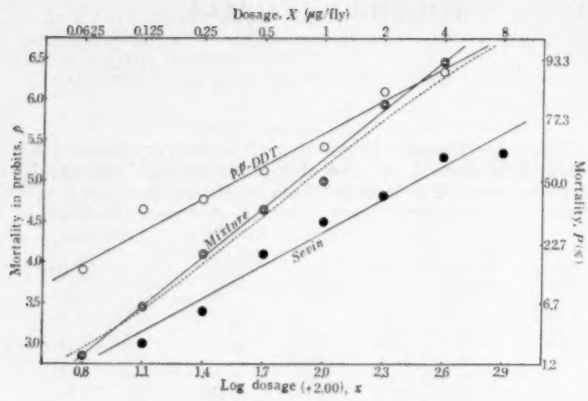

FIG. 1.—Dosage-mortality regression lines for Sevin, *p,p'*-DDT, and their 2 to 1 combinations applied topically in acetone solution to female adults of the house fly, *Musca domestica*. Dotted line is from equation $y=a_1+b \log \{\rho_2+(1-\rho_2)\ 10^{-kX}\}+b \log X$ for $\rho_2=8.623$ and $k=0.0025$.

two insecticides are said to act similarly. In this equation, a_1 is the parameter of position of the first insecticide, b is the combined slope, and ρ_2 is the potency of the second insecticide relative to that of the first. In such a case, the ld-p line for the mixture is parallel to that of the first and the second insecticides. However, in case the slope of the ld-p line for the mixture is not the same as that of each insecticide, as in the present instance, it is necessary to set up a further mathematical model of what might occur when two insecticides which show similar action are combined. Under conditions that make the proportionate constitution of the mixture dependent upon the total concentration, a study has been made of the behavior of a mixture in the proportions,

$$\pi_1=1-\pi_2=10^{-kX}.$$

Here k is a positive constant; the law is not intended as even an approximation to any physico-chemical relationship, but has simply been chosen so that the proportion of the first concentration, π_1, is zero at high concentrations and changes continuously to 1 at low concentrations. With this substitution for π_1, the previous equation may be written as

$$y=a_1+b \log \{\rho_2+(1-\rho_2)10^{-kX}\}+b \log X.$$

In a previous paragraph, it has been shown that the ld-p lines for Sevin and *p,p'*-DDT are parallel within the experimental error when the chemicals are applied separately to the house flies. The combined slope of these two ld-p lines as calculated from the data for *p,p'*-DDT and Sevin (table 1) is,

Table 3.—Comparison of the observed mortalities for the mixture with the predictions calculated with the equation $y=1.871+1.258 \log(8.623-7.523\times10^{-0.0025X})+1.258 \log X$.

Dosage of Mixture (µg./Fly) ×100	*p,p'*-DDT Equivalent Concentration	Observed Probit	Expected Probit	Weight	χ^2
6.25	2.57	2.8556	3.0030	16.65	0.3618
12.5	5.13	3.4452	3.3765	26.13	0.1233
25	10.27	4.1073	4.0145	51.32	0.4418
50	20.53	4.6415	4.5916	94.28	0.2348
100	41.07	5.0050	5.1888	73.41	2.4797
200	82.13	5.9502	5.7639	54.28	1.8841
400	164.27	6.4833	6.2712	40.31	1.8135

$$b=1.258.$$

The probits y_s, y_d of the per cent mortality resulting from dosage X_s, X_d of Sevin and *p,p'*-DDT, respectively, may be predicted from

$$y_s=1.871+1.258 \log X_s$$
$$y_d=3.048+1.258 \log X_d.$$

Relative potency of *p,p'*-DDT to Sevin, calculated by use of this combined slope, is $\rho_d=8.623$, which is almost exactly that calculated earlier from the separate ld-p lines.

In table 3, the observed mortalities for the mixture are compared with predictions calculated with $\rho^2=8.623$, $k=0.0025$ and the equation for Sevin. That is, expected probits shown in the fourth column were calculated with the equation,

$$y=1.871+1.258 \log (8.623-7.623\times10^{-0.0025X}) +1.258 \log X.$$

The value of k has been chosen as roughly corresponding to the data shown in table 1. Ratios of two insecticides will affect only the value of k.

A test of the agreement between hypothesis and observation can be made by finding the weights to be associated with the expected probits and multiplying these by the squares of the corresponding discrepancies, and adding to give a χ^2 with seven degrees of freedom. It is found that

$$\chi^2=7.339<14.067(n=7)$$

is a value which could easily occur as a result of chance deviations.

In this type of joint action, it can be considered that Sevin and *p,p'*-DDT have different types of toxic action, and the toxic action of the mixture is owing chiefly to the more effective component, *i.e.*, *p,p'*-DDT when low concentrations are used and the effect of the other component, Sevin, is most noticeable at high concentrations when the effect of the *p,p'*-DDT has approached a maximum. It is considered that the effect of the mixture will

Table 2.—Summary of experiments on the toxicities of Sevin, *p,p'*-DDT, and their 2 to 1 combinations applied topically in acetone solution of female adults of *Musca domestica*.

Toxicant	No. of Insects	Regression Equation $y=\bar{y}+b(x-\bar{x})$	χ^2	Variance of Parameter y $V(\bar{y})$	Variance of Parameter b $V(b)$
Sevin	806	$y=4.64693+1.238\,(x-2.20643)$	$9.007<11.070\,(n=5)$	0.00280	0.01087
p,p'-DDT	809	$y=5.16341+1.273\,(x-1.68128)$	$7.777<11.070\,(n=5)$	0.00244	0.00807
Mixture	856	$y=4.84481+1.974\,(x-1.81102)$	$5.131<11.070\,(n=5)$	0.00290	0.01409

depend upon the total concentration of the insecticide, and as the concentration increases the proportionate toxic action of Sevin to *p,p'*-DDT will change smoothly from 0 to 1.

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Field Experiments with Several Insecticidal Sprays Against the Boll Weevil and Bollworm[1]

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ABSTRACT

In experiments at Stoneville, Mississippi, in 1957 and 1958 a Guthion-DDT mixture gave outstanding control of the boll weevil (*Anthonomus grandis* Boh.). Sevin® (1-naphthyl methylcarbamate), toxaphene-malathion, dicapthon, Bayer 29493 (*0,0*-dimethyl *0*-(4-methylthio-*m*-tolyl) phosphorothioate), and mixtures of Monsanto CP 7769 (hexaethyl (ethylthiomethylidyne) triphosphonate), methyl parathion, toxaphene, or malathion with DDT gave generally satisfactory control, but Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide), ronnel-DDT, toxaphene, and endrin were not effective. The mixture of DDT with ronnel, Monsanto CP 7769, Guthion® (*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3-(4*H*)-ylmethyl) phosphorodithioate), methyl parathion, and malathion were effective in controlling the bollworm (*Heliothis zea* (Boddie)).

Field-plot experiments were conducted in the vicinity of Stoneville, Mississippi, in 1957 and 1958 to determine the effectiveness of several insecticides against the boll weevil (*Anthonomus grandis* Boh.) and the bollworm (*Heliothis zea* (Boddie)). A field-cage experiment was also conducted against the boll weevil in 1957.

In 10 field-plot experiments treatments were replicated three or four times in randomized blocks. The size of the plots ranged from 0.2 to 2.5 acres. In most of the experiments 6 to 6.5 gallons of spray per acre were applied with a high-clearance sprayer, but in Experiment 2 a tractor-mounted sprayer was used to apply about 3.8 gallons per acre. Three nozzles per row were used with both sprayers. The application interval ranged from 3 to 10 days. All the insecticide formulations were emulsifiable concentrates, except Sevin® (1-naphthyl methylcarbamate) which was a dispersed solid. Infestation records were made about weekly on the eight middle rows by examining 100 squares, blooms, or bolls at one or more points in each plot. Yields were obtained from the 12 middle rows.

In Experiments 1, 2, and 4 wet field conditions prevented the use of ground spraying equipment, and so several blanket aerial applications were made to the entire field.

The boll weevil was the major economic pest in most of the experiments. Damage by the bollworm was light in 1957, but considerable damage occurred in 1958, particularly in early September. Other cotton pests were not present in sufficient numbers in either season to cause much damage.

In the plots where Experiment 2 was conducted, boll weevils were also caged on plant terminals in each treatment at several intervals following a regular tractor-application of the insecticides on August 5. The cages were half-pint ice-cream cartons in which the tops and bottoms had been replaced with wire screen. Mortality records were made at 24-hour intervals until the next treatment.

The results of these experiments are summarized in tables 1, 2, 3, and 4.

The following treatments gave generally satisfactory control of the boll weevil and bollworm and most of them increased yields over the untreated check: Guthion-DDT, Sevin, Monsanto CP 7769-DDT, methyl parathion-DDT, toxaphene-malathion, toxaphene-DDT, malathion-DDT, and malathion-endrin.

Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide) ronnel-DDT, toxaphene, and endrin were generally unsatisfactory for control of the boll weevil.

Where bollworms are a problem, DDT should be added to ronnel, Monsanto CP 7769 (hexaethyl (ethylthiomethylidyne) triphosphonate), Guthion® (*O,O*-dimethyl-*S*-(4-oxo-1,2,3-benzotriazin-3-(4*H*)-ylmethyl) phosphorodithioate), methyl parathion, dicapthon, Bayer 25141 (*O,O*-diethyl *O*-(*p*-methylsulfinyl) phenyl phosphorothio-

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[2] In cooperation with the Mississippi Agricultural Experiment Station.

Table 1.—Effectiveness of several insecticides applied as sprays against the boll weevil and bollworm.

Insecticide		Per Cent of			Seed Cotton per Acre (Lb.)	
		Weevil-punctured Squares	Bollworm-injured			
Pounds per Gallon	Pounds per Acre		Squares	Bolls	Total	Gain Over Check
Experiment 1 (7 applications August 3 to September 9, 1957)[a]						
Thiodan 2	0.5	47	2.2	1.0	1,365	290
Ronnel 2+DDT 2	1.0 +0.5	42	1.1	1.4	1,365	290
Monsanto CP 7769 2+DDT 2	0.5 +0.5	26	1.5	0.5	1,470	395
Sevin 1	2.0	19	1.6	.7	1,350	275
Guthion 1.5+DDT 2	0.25+0.5	12	0.9	.7	1,590	515
Untreated	—	47	3.7	1.8	1,075	—
L.S.D. at 5% level	—	10	*	*	214	—
Experiment 2 (8 applications July 29 to September 2, 1957)[b]						
Thiodan 2	0.5	54	0.5	2.3	1,823	− 65
Ronnel 2+DDT 2	1.0 +0.5	29	.6	2.2	1,743	−145
Monsanto CP 7769 2+DDT 2	0.5 +0.5	27	1.6	1.4	2,093	205
Sevin 1	2.0	20	1.1	1.2	1,850	− 38
Guthion 1.5+DDT 2	0.25+0.5	14	0.8	0.9	1,900	12
Untreated	—	60	2.6	1.3	1,888	—
L.S.D. at 5% level	—	17	1.3	*	*	—
Experiment 3 (16 applications June 18 to September 10, 1957)						
Toxaphene 6	4.0[c]	45	2.4	0.9	1,070	370
Toxaphene 6+malathion 5	2.0 +0.5	35	1.7	1.5	1,500	800
Methyl parathion 2+DDT 2	0.25+0.5	35	1.4	0.5	1,545	845
Untreated	—	56	2.5	1.8	700	—
L.S.D. at 5% level	—	12	*	*	449	—
Experiment 4 (10 applications July 5 to September 9, 1957)[a]						
Toxaphene 6	4.0	41.4	2.1	0.2	1,800	725
Toxaphene 6+malathion 5	2.0 +0.5	13.4	1.1	.5	1,600	525
Methyl parathion 2+DDT 2	0.25+0.5	19.6	1.0	.3	1,810	735
Untreated	—	46.8	4.2	1.8	1,075	—
L.S.D. at 5% level	—	6.8	*	*	*	—
Experiment 5 (7 applications August 11 to September 8, 1958)						
Dicapthon 2	1.0	11	4.0	4.2	1,425	122
Sevin 2.5	1.0	10	1.8	2.1	1,796	493
Hercules 5727[d] 1.5	1.5	16	3.7	1.6	1,545	242
Sevin 4	1.0	12	2.1	1.2	1,839	536
Bayer 25141 2	0.25	15	2.4	2.6	1,826	523
Bayer 29493 2	0.5	9	4.8	3.4	1,748	445
Guthion 1.5+DDT 2[e]	0.25+1.0	6	1.5	0.9	2,198	895
Untreated	—	30	5.0	4.7	1,303	—
L.S.D. at 5% level	—	*	2.1	2.3	312	—
Experiment 6 (7 applications August 13 to September 10, 1958)						
Toxaphene 6	3.0	39	2.2	1.2	1,933	463
Toxaphene 4+DDT 2	2.0 +1.0	6	2.0	0.4	2,108	638
Malathion 5+DDT 2[e]	0.5 +1.0	15	2.0	1.4	2,184	714
Methyl parathion 2+DDT 2	0.25+0.25	12	1.8	0.6	2,177	707
Guthion 1.5+DDT 2[e]	0.25+1.0	5	2.8	.8	2,284	814
Untreated	—	41	4.5	2.0	1,470	—
L.S.D. at 5% level	—	14	1.5	1.0	311	—

[a] Airplane applications of calcium arsenate plus 5% DDT on Sept. 4 and 14 and of 5% malathion plus 10% DDT on Sept. 18.
[b] Airplane spray applications of Guthion at 0.25 lb. per acre on July 24 and at 0.5 lb. on Sept. 5.
[c] 3 lb. for first 2 applications; parathion at 0.1 lb. on Aug. 16 for aphids.
[d] Carbamic acid, methyl-*m*-isopropylphenyl ester; first application on Aug. 20.
[e] DDT included only in alternate applications.
* Not significant according to *F* test.

Table 2.—Effectiveness of several insecticidal sprays at different dosages and intervals of treatment against the boll weevil and bollworm—1957.

Insecticide		Days Between Treatments	Per Cent of			Seed Cotton per Acre (Lb.)	
			Weevil-punctured Squares	Bollworm-injured			
Pounds per Gallon	Pounds per Acre			Squares	Bolls	Total	Gain Over Check
Experiment 7 (Applications August 9 to September 14)							
Malathion 5+DDT 2	0.5 +0.5	3	32	0.9	1.1	1,730	267
	1.0 +0.5	3	20	.4	1.5	1,803	340
		5	36	.6	0.8	1,783	320
	2.0 +0.5	5	34	.5	.6	1,813	350
Malathion 5+endrin 1.6	0.5 +0.2	5	41	.7	1.4	1,845	382
Endrin 1.6	.3	5	50	.9	0.9	2,005	542
Guthion 1.5+DDT 2	0.1 +0.5	3[a]	33	1.9	3.4	1,870	407
	0.25+0.5	5	27	1.0	1.4	1,988	525
Untreated	—	—	58	1.8	5.1	1,463	—
L.S.D. at 5% level	—	—	14	1.0	2.6	*	—
Experiment 8 (Applications July 19 to September 10)							
Methyl parathion 2+DDT 2	0.25+0.5	3	18	1.8	1.3	1,145	430
		5	23	0.9	1.5	1,088	373
	0.5 +0.5	3	12	.3	0.9	1,263	548
		5	17	1.2	2.8	1,228	513
Guthion 1.5+DDT 2	0.25+0.5	5	14	0.9	3.2	1,588	873
Untreated	—	—	43	2.2	5.0	715	—
L.S.D. at 5% level	—	—	16	1.2	*	486	—
Experiment 9 (Applications August 1 to September 10)							
Guthion 1.5+DDT 2	0.25+0.5	5	15	0.5	1.2	—	
		7	25	0.9	0.3	—	
	0.5 +0.5	7	17	0.5	0.9	—	
		10	27	0.7	1.3	—	
	0.75+0.5	10	20	1.1	2.2	—	
Untreated	—	—	52	3.4	4.4	—	
L.S.D. at 5% level	—	—	9	1.7	1.8	—	

[a] First application on August 15; DDT included in alternate applications only.
* Not significant according to the *F* test.

Table 3.—Effectiveness of several insecticides applied as sprays against the bollworm, 1958.

Insecticide		Per Cent of Bollworm-Injured Bolls		No. of Live Larvae per 100	
Pounds per Gallon	Pounds per Acre	Sept. 2	Sept. 8	Blooms Sept. 2	Bolls Sept. 8
Experiment 10 (Applications August 8, 14, 28)					
DDT 2	1.0	0.7	0.7	0	0
Endrin 1.6	0.3	1.0	0.7	0	0.7
Toxaphene 6	3.0	0.7	6.7	0.3	2.7
Toxaphene 4 +DDT 2	2.0+1.0	1.0	2.7	0	2.0
Sevin 2.5	1.0	2.0	1.3	0	0
	2.0	0.7	0.7	0	0
Untreated	—	3.3	16.7	3.0	12.9
L.S.D. at 5% level	—	1.4	7.8	0.4	7.2

Table 4.—Per cent control of caged boll weevils after treatment with different insecticides, 1957.[a]

Insecticide (Lb./A.)	Caged Immediately				Caged After 24 Hours			Caged After 48 Hours	
	24 Hours	48 Hours	72 Hours	96 Hours	24 Hours	48 Hours	72 Hours	24 Hours	48 Hours
Thiodan 0.5	24	37	33	40	9	12	20	3	4
Ronnel 1.0+DDT 0.5	27	50	62	70	4	37	61	14	30
Monsanto CP 7769 0.5+DDT 0.5	58	94	100	—	64	89	93	29	47
Sevin 2.0	68	90	92	92	53	89	100	45	70
Guthion 0.25+DDT 0.5	100	—	—	—	100	—	—	100	—

[a] Sprays applied August 5.

ate), Bayer 29493 (*O,O*-dimethyl *O*-(4-methylthio-*m*-tolyl) phosphorothioate), and malathion.

The residual effectiveness of Guthion-DDT against the boll weevil was considerably greater than that of Thiodan, ronnel-DDT, Monsanto CP 7769-DDT, and Sevin.

Observations of Feeding Habits of the Spotted Alfalfa Aphid on Resistant and Susceptible Alfalfa Plants[1]

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ABSTRACT

Spotted alfalfa aphids (*Therioaphis maculata* (Buckton)) placed on both resistant and susceptible alfalfa plants soon assumed a feeding position. On resistant plants, they became restless in 1 to 4 hours and eventually died or left the plants. Little or no honeydew was produced on resistant plants whereas profuse honeydew production occurred on susceptible plants. Survival of aphids placed first on resistant plants and later transferred to susceptible plants was as good as the survival of aphids transferred from susceptible plants, indicating no residual toxic effect from the resistant plants. Aphids confined to highly resistant plants were found to die at nearly the same rate as aphids confined with no source of food. Microscopic examination of leaf sections indicated that phloem tissue is the principal feeding site. However, it was observed that the setal tubes entered the phloem less frequently in resistant than in susceptible plants, suggesting that the aphid's mechanism for locating the phloem was interfered with in resistant plants. Results of these studies indicate that mortality of the spotted alfalfa aphid on highly resistant plants is caused by starvation or desiccation resulting from a failure to ingest a sufficient quantity of plant sap.

In studies of alfalfa resistant to the spotted alfalfa aphid, (*Therioaphis maculata* (Buckton)), both tolerance and antibiosis have been observed (Howe & Smith 1957). Antibiosis has been commonly used as a criterion for the final selection of plants in breeding programs. The true nature of the antibiosis has not been reported. On highly resistant alfalfa plants such as C-84, aphids confined to the plants usually die in a period of 24 to 72 hours. Death might be caused by a toxic material in the plant, to a nutritional deficiency in the imbibed juice or to a failure to feed, resulting in starvation or desiccation of the aphids. Observations and experiments reported herein indicate that failure to feed is the cause of death of the aphids. Further studies are necessary to determine the nature of the mechanism which prevents feeding.

BEHAVIOR OF APHIDS ON HIGHLY RESISTANT PLANTS.—Howe & Smith (1957), in studying resistance of Lahontan alfalfa to the spotted alfalfa aphid, observed that aphids introduced on parental clones C-84, C-89, and C-900 died within 48 hours after introduction, and that nymphs born on these clones were restless and tended to scatter over the leaflets of the plants.

A close observation of the behavior of individual aphids on clone C-84 was carried out in the laboratory. It was noted that aphids introduced onto these plants soon settled down as they would on susceptible plants, and from outward indications, appeared to be feeding. They usually remained in this position for a period of 1 to 4 hours, but then began wandering around on the plant. After a few minutes, however, most aphids again settled and appeared to commence feeding. This behavior pattern was repeated several times over a period of 8 to 36 hours, until restlessness increased and aphids crawled off of the plant or died. In contrast, aphids on susceptible plants usually remained in their initial feeding position for an indefinite period unless disturbed. Aphids introduced onto resistant plants gradually became smaller or shrunken in appearance.

Aphids confined under gelatin capsules on leaves of C-84 produced no observable honeydew. In contrast, aphids on susceptible plants deposited large amounts of honeydew against the sides of gelatin capsules. This is in

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agreement with studies of Maxwell & Painter (1959).

EFFECTS OF TRANSFERRING APHIDS FROM RESISTANT TO SUSCEPTIBLE PLANTS.—Tests were carried out in the greenhouse at temperatures of 70° to 80° F. Alate female spotted alfalfa aphids were confined on leaves of C-84 and on susceptible Caliverde plants by means of gelatin capsules stuck to the leaf surface with saliva. After a period of 32 hours, death of some aphids on C-84 leaves had occurred. At this time, one-half of the remaining live aphids were transferred to Caliverde leaves and confined in the same manner. To serve as a check, to indicate whether any mortality would result from transferring alone, one-half of the aphids on the Caliverde plants were also transferred to leaves of other Caliverde plants. Results are shown in table 1.

The results indicated that aphids transferred from C-84 to Caliverde plants survived essentially as well as aphids transferred from Caliverde to Caliverde plants. Thus aphids confined on C-84 were able to recover and resume reproduction if subsequently given a susceptible plant as a source of food.

COMPARISON OF SURVIVAL OF APHIDS ON C-84 AND APHIDS HAVING NO SOURCE OF FOOD.—Aphids were confined in cylindrical cages 1⅛ in. in diameter by 1¼ in. long, which were made from plastic vials. The top of the cylinder was covered with nylon screen and the bottom was sealed by a 2- × 2-in. glass plate held in position by a rubber band. Leaves on a potted C-84 plant were caged by inserting the petiole between the cylinder and the glass plate, so that an entire trifoliate leaf was contained in the cage. The cage was then suspended by a string attached to a stake inserted in the soil. Ten adult apterous spotted alfalfa aphids were introduced into each of five cages. The same number of aphids were introduced into five additional cages containing no plant material, so that death of these aphids would be by starvation or desiccation. Counts of live adults and approximate numbers of young produced were taken at various intervals. Two different tests were performed, one being in a laboratory room having a temperature of approximately 77° F. and the other in a plant growth chamber at approximately 95° F. Results are shown in table 2.

Results of the 77° F. test indicate that mortality of adult aphids occurred at essentially the same rate in aphids having access to C-84 leaves and in aphids having no food source. Rate of producing young also appeared very similar in both cases.

Results of the 95° F. test show an increased rate of mortality and a decrease in production of young compared with the 77° test. In this case, however, aphids on C-84 leaves survived somewhat longer and produced more young than aphids confined in the empty cages. It was not determined why this occurred at 95° but not at 77° F. At least two factors might be responsible. Most likely is the possibility of higher humidity in the cages containing leaves. At 77° this might not have been so critical, but at 95° the higher humidity might have materially reduced the rate of desiccation of the aphid, and hence increased the length of life. Another factor which might effect the differential survival rate is a possible change in the physiology of the resistant plant at temperatures as high as 95° F., so that resistance is not complete. That is, the plant may change so that the aphid is able to do a slight amount of feeding at 95° and thus prolong its life relative to the nonfeeding aphids.

Table 1.—Effects of transferring aphids between resistant and susceptible plants on aphid survival.

PLANT FIRST CONFINED ON	PLANT TRANSFERRED TO	TOTAL APHIDS	NO. SURVIVING	SURVIVAL (%)
C-84	Caliverde	12	10	83.3
	Caliverde	8	7	87.5
	Not transferred	12	0	0
	Not transferred	9	0	0
Caliverde	Caliverde	12	10	83.3
	Caliverde	9	9	100
	Not transferred	12	11	91.7
	Not transferred	9	9	100

Table 2.—Survival and reproduction of 50 aphids confined on resistant plants or in empty leaf cages.

Test 1. 77° F.

HOST PLANT	LIVE ADULTS			YOUNG PRODUCED	
	18 Hrs.	24 Hrs.	42 Hrs.	18 Hrs.	24 Hrs.
C-84	36	24	1	134	166
None	41	22	0	142	168

Test 2. 95° F.

HOST PLANT	LIVE ADULTS				YOUNG PRODUCED
	16 Hrs.	21 Hrs.	24 Hrs.	42 Hrs.	16 Hrs.
C-84	26	11	5	1	80
None	20	6	1	0	35

MICROSCOPIC EXAMINATION OF ALFALFA LEAF SECTIONS CONTAINING APHID STYLET SHEATHS.—Trifoliate leaves of C-84 and Caliverde plants were artificially infested with large numbers of spotted alfalfa aphids. In investigations carried out in 1956, the plants were grown in the greenhouse at temperatures of 70° to 80° F. before aphids were introduced. In the 1958 studies, plants were kept at a constant temperature of 85° F. in a plant growth chamber for a period of 6 days before introduction of aphids. At this temperature the resistance of C-84 to the aphid is complete.

Leaves upon which aphids had been introduced were killed, dehydrated, embedded and sectioned according to standard histological techniques, and the sections were stained with a safranin 0 and fast green combination.

No consistent anatomical differences were noted between leaflets of resistant and susceptible plants. In general, C-84 leaflets were thicker in cross-section than those of Caliverde; greater thickness apparently being due to longer palisade parenchyma cells. However in the progeny of a C-84×Caliverde cross there was no relationship between leaf thickness and resistance to the aphid. Thus the association of the two characters in the parent is believed to be one of chance.

The salivary sheath formed around the aphid stylets inserted in the plant is permanent, and thus can be used to study the course taken by the stylets, without actually

Table 3.—Frequency of stylet sheaths entering vascular bundles.

Plant	No. Sheaths		Per Cent Entering Bundle
	In Bundle	Not in Bundle	
C-84, 1956	24	36	40.0
C-84, 1958	22	46	32.4
Total	46	82	35.9
Caliverde, 1956	42	24	62.1
Caliverde, 1958	49	25	66.2
Total	91	49	65.0

having the latter fixed in the plant tissue. Therefore it was not felt necessary to attempt to fix aphids in place on the leaves.

Stylet Sheaths in Caliverde Leaflets.—General observations were similar to those described by Diehl & Chatters (1956). Their studies showed considerable feeding in the mesophyll. In the present studies, however, it appeared that the vascular bundles of the leaf were definitely the principal sites of feeding. As shown in table 3, considerably more stylet sheaths terminated in the vascular bundles than in the mesophyll area. It was observed that stylet sheaths often showed that the aphid mouthparts made a sharp change in direction, sometimes as much as a 45° angle, and proceeded directly into a vascular bundle. Other stylet sheaths contained several branches, with one branch often terminating in a vascular bundle. If there was no vascular tissue in the vicinity of penetration, the sheaths often proceeded in a straight line with no branching. If penetration had taken place near a vascular bundle, however, the stylet sheath nearly always terminated in the bundle. It appeared that the sheaths which entered the vascular tissue almost invariably terminated in the phloem. Damage to mesophyll cells was observed occasionally.

Stylet Sheaths in C-84 Leaflets.—As shown in table 3, vascular bundles of C-84 leaflets were reached less frequently than on Caliverde leaflets. Many of the stylet sheaths observed indicated that the aphid stylets passed close to a vascular bundle, but continued on in their original direction without entering into the bundle. Some appeared to be headed directly toward a bundle but then changed direction before entering it. Some stylet sheaths passing as close as 3 to 5 microns to a vascular bundle failed to enter. In comparison, observations on Caliverde leaflets revealed that the closest distance a stylet sheath passed by a bundle without entering was 13 microns, while most were found to be greater than 30 microns.

The stylet sheaths which passed into the vascular tissue did not always terminate in the phloem. Sometimes it was observed that a sheath actually passed through the phloem tissue and terminated outside of the vascular bundle or in the xylem area. In a few other cases, sheaths entered directly into the xylem and terminated or continued out of the bundle entirely. This was not observed in Caliverde leaflets.

Discussion.—From the above studies, it would appear that spotted alfalfa aphids do little or no feeding on highly resistant plants such as the C-84 clone at warm temperatures. This is indicated by lack of honeydew production, tendency of the aphids to become restless, and the shrunken appearance of aphids.

Although the possibility that a substance toxic to spotted alfalfa aphids as the basis for resistance cannot be definitely excluded, it does not appear likely on the basis of the present evidence. In transferring aphids from resistant C-84 plants to susceptible Caliverde plants, it might be expected that some aphids would fail to survive even when returned to a susceptible plant as a result of the residual toxin in the aphid if toxins were taken up from the resistant plant.

Evidence that aphids on C-84 plants at 77° and 95° F. die in nearly the same period of time as starved aphids also suggests that a toxic substance is not involved, as then it might be expected that death of poisoned aphids would occur either more or less rapidly than death by starvation, depending on the degree of toxicity. From the results of this test, it would appear that death is caused by starvation or desiccation. The shrunken appearance of aphids both on C-84 plants and in empty cages is consistent with this idea.

The microscopic examinations of leaf sections show that aphids are able to insert their stylets and penetrate the vascular bundles on highly resistant C-84 plants, suggesting that the basis for resistance does not lie in structural differences in the plants. This hypothesis is further supported by results of investigations carried out on the effects of temperature on resistance. It has been demonstrated that resistance to the spotted alfalfa aphid is expressed to a lesser degree at cool temperatures (Hackerott & Harvey 1959). Work conducted at this station indicates that the altering of the expression of resistance is caused mainly by a change in the plant itself, and that this change takes place within a period as short as 2 days when plants are moved from one temperature to another. Such a rapid change in leaves that are already mature would indicate that resistance is of a physiological rather than of a morphological nature.

From examinations of aphid stylet sheaths, it appears that the aphids were quite efficient in reaching vascular bundles on Caliverde leaves, but much less so on C-84 leaves. Thus it would seem that the aphid's mechanism of locating or perceiving the phloem tissue is in some way disrupted on C-84 plants. This conceivably could account for a failure of the aphid to take up large amounts of sap from the plant. However, stylet sheaths actually terminated in phloem tissue in enough cases so that the very high degree of resistance of C-84 could not be fully explained on the basis that aphids are unable to reach the phloem. Therefore it would seem that even those aphids that reach the phloem on C-84 are unable to feed normally and survive. Whether this could be caused by a nonpreference reaction of the aphid, or to the aphid actually being incapable of taking up plant sap normally, has not been determined.

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The Influence of Insecticide-Fungicide Spray Programs on the Growth of Apple Nursery Trees[1]

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ABSTRACT

Applications each year over a 3-year period of four bi-weekly sprays, containing insecticides and fungicides, to apple nursery trees increased tree vigor and tree grade considerably. Sulfur was the most effective fungicide, while demeton-DDT or lindane-DDT provided excellent insect control. The effects of adequate insect and disease control appeared to be cumulative over the 3-year period. Considerable differences were evident in trees of different apple varieties in response to adequate insect and disease control as indicated in tree growth. Less vigorous apple varieties showed a greater benefit from spraying than the more vigorous varieties, but significant gains from spraying were obtained with all varieties.

The economic value of apple trees in nursery plantings is determined primarily by their relative size and freedom from injurious insects and plant diseases. Pests that may affect the growth and quality of apple nursery stock include potato leafhopper (*Empoasca fabae* (Harr.)), apple aphid (*Aphis pomi* DeG.), woolly apple aphid (*Eriosoma lanigerum* (Hausm.)), tarnished plant bug (*Lygus lineolaris* (P. de B.)), and powdery mildew (*Podosphaera leucotrichia* (E. & E.) Salm.).

Previous studies have shown that DDT is highly effective against the potato leafhopper (Gambrell 1948, Gambrell & Gilmer 1956); also parathion, malathion, BHC, lindane, and TEPP have shown promising results against the apple and woolly apple aphids (Gambrell & Young 1950, Gambrell & Gilmer 1956). The current study was undertaken to determine the influence of the aforementioned pests on growth and quality of apple trees and to develop satisfactory measures for their control.

MATERIALS AND METHODS.—The experimental design was a 4×4 Latin square in which individual plots consisted of four rows 100 feet long spaced 3½ feet apart. Alleys, 10 feet wide, between plots and between replications minimized the effects of spray drift.

A total of 9,558 seedling apple rootstocks were planted in the 16 plots during April, 1956. In July, 1956, each row in the individual plots was budded to a different apple scion variety; consequently, during 1957 and 1958, each plot contained four apple varieties. Idared and Monroe were selected because of their pronounced susceptibility to powdery mildew while Roger's McIntosh and Golden Delicious were included because of their susceptibility to the potato leafhopper.

Sprays were applied with a handgun attached to a hydraulic sprayer operating at pressures of 300 to 400 p.s.i. Gallonage per acre increased from about 75 gallons, applied to apple rootstocks in 1956, to about 125 gallons applied to the 1-year budlings in 1957 and finally, to about 200 gallons applied to 2-year trees in 1958. Each plot received the same materials at the same rates per 100 gallons throughout the 3-year period of growth.

Insecticides tested were lindane, demeton, and malathion; fungicides included sulfur, copper-sulfur-lime, and Karathane® (2-(1-methylheptyl)-4,6-dinitrophenyl crotonate). Each insecticide and fungicide was used singly and in all possible combinations of a single insecticide and a single fungicide. DDT was added to all single insecticides, single fungicides, and insecticide-fungicide combinations for leafhopper control. One plot received no insecticides or fungicides and served as an unsprayed control.

In 1956, three sprays were applied at biweekly intervals, beginning June 20 and terminating July 27. Five biweekly sprays were applied in 1957, beginning May 28 and terminating August 12; similarly, 5 biweekly sprays were applied in 1958, beginning May 20 and terminating July 31. Insecticides were omitted in the initial fungicide applications made in 1957 and 1958 on the 1- and 2-year trees.

Rates of materials per 100 gallons were as follows in all years: 25% lindane (W.P.) 1 lb.; 62% demeton (emulsion) 1 pt.; 25% malathion (W.P.) 2 lbs.; 50% DDT (W.P.) 2 lbs.; sulfur 5 lbs.; copper-sulfur (33% metallic Cu as basic sulfate and sulfur in a 1:1 ratio) 1½ lbs. plus lime 1½ lbs.; 22.5% Karathane (W.P.) ¾ lb. A wetting agent (B-1956) was added at the rate of 4 oz. per 100 gallons to all sprays.

RESULTS.—Infestations of apple aphid were relatively light in 1956 and 1957 and were controlled readily in all plots receiving lindane, demeton, and malathion. Very heavy infestations of this insect developed in 1958, a season of copious and well-distributed rainfall which favored vigorous terminal growth of the trees. Infestation was heavy by mid-June and continued to develop through July and early August; populations were consistently higher than at any time in the previous 10 years. Even the superior treatment, demeton-DDT, failed to prevent reinfestations and population buildup when applied in a biweekly schedule. Infestations in treatments which received no effective aphicide and in the unsprayed control were amply severe to stop terminal growth. Of the aphicidal materials, demeton was slightly superior to lindane,

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Table 1.—Apple aphid control reflected as mean percentage of infested terminals on 2-year apple nursery trees at various time intervals after spraying.

Insecticide	Terminals Infested on Indicated Days After Initial Application[a] (%)		
	7	14	22[b]
Lindane+DDT	25	69	6
Demeton+DDT	11	53	2
Malathion+DDT	34	75	42
DDT	70	96	97
Untreated	86	100	100

[a] Records taken following spray application of June 26.
[b] A second spray application was made on July 15, 3 days before counts were taken.

while malathion was relatively ineffective under these severe conditions. DDT alone gave no significant degree of aphid control (table 1).

As is normal for western New York, heavy infestations of potato leafhopper developed in the unsprayed control each year of the experiment. Because of the very rapid influx of adults into all plots from nearby commercial plantings, the data obtained in the plots were confined to counts of nymphal stages only. Representative data from 1957 are presented in table 2. DDT alone, or in combination with any of the three aphicides, gave total kill of nymphs 1 day after applications were made. Counts made 28 days after application demonstrated a 70% reduction in nymphal population in the demeton-DDT plots, possibly indicating some persistent systemic action; however, this degree of control could not be considered entirely satisfactory. A noticeable reduction in nymphal populations in the lindane-DDT and malathion-DDT plots was also evident 28 days after application, but DDT alone provided very little control of nymphs 28 days after application (table 2).

Woolly apple aphid infestations over the 3-year period were too light and scattered to obtain reliable data on root injury.

Effect of Treatments on Growth.—Height and caliper (trunk diameter) of individual trees were measured both in 1957 when the trees were 1-year budlings and in 1958, immediately after the 2-year trees were harvested. Grade classes for 1-year and 2-year trees are given in table 3. In 1958 records were obtained on 6,427 2-year trees at harvest.

Table 2.—Control of potato leafhopper in 1-year apple nursery trees with various insecticides.

Insecticide	Reduction in Nymphs on Indicated Days After Application[a] (%)	
	1	28
Demeton+DDT	100	70
Malathion+DDT	100	44
Lindane+DDT	100	32
DDT	100	7
Untreated[b]	0	0

[a] Sprays containing wettable sulfur and insecticides shown were applied June 13, 1957. 1-day counts made June 14; 28-day counts made July 11.
[b] Plots receiving no insecticide averaged 16.5 nymphs per terminal on July 11.

Table 3.—Classification of tree grades for 1-year and 2-year apple nursery trees.

Grade	Caliper[a]	Height (Inches)
	1-year trees	
1	9 or above	42 or above
2	7–9	36
3[b]	Below 7	Below 36
	2-year trees	
Extra	13 or above	Above 48
1	11–13	Above 48
2	9–11	Above 42
3[b]	Below 9	Below 42

[a] Trunk diameter at a point 3 inches above the scion union expressed as 1/16 inch units.
[b] Normally, there is little commercial demand for trees in Grade 3.

The individual plots averaged between 325 and 400 trees, so that 80 to 100 trees of each of the four varieties were included in each treatment. As shown in table 4, the principal factors influencing tree grade were the fungicide most effective in controlling powdery mildew and the use of DDT for control of the potato leafhopper. Excellent powdery mildew control was obtained in plots receiving sulfur; trees in these plots not only possessed superior foliage color and general appearance, but they also made the most vigorous growth and best tree grade. Karathane, while it afforded good control of powdery mildew, caused some marginal leaf injury and leaf mottling in both 1957 and 1958. Although the degree of injury appeared to be relatively slight, it may have been sufficient to account for the reduced vigor and lower grades of trees evident in the Karathane plots. Mildew control with copper-sulfur-lime was not highly satisfactory, even though trees in these plots ranked second in tree vigor and tree grade.

All of the aphicide-DDT combinations performed adequately. The demeton-DDT plots were equal or superior to either lindane-DDT or malathion-DDT plots in the percentage of grade 1 or above trees. Even where no fungicide was added to the aphicide-DDT combinations the percentage of trees of grade 1 or above was double that of the unsprayed control (table 4).

Table 4.—Effects of insecticide-fungicide combinations on caliper and grade of 1-year and 2-year apple nursery trees of four varieties 1957 and 1958, respectively.

Fungicide	Insecticide	Mean Caliper[a]		Percentage in Grade 1 or Above	
		1-Year	2-Year	1-Year	2-Year
Sulfur	Lindane+DDT	8.7	13.6	60	95
	Demeton+DDT	8.9	13.9	60	92
	Malathion+DDT	8.7	13.1	60	93
	DDT	9.0	13.5	57	93
Copper-sulfur	Lindane+DDT	8.1	12.4	33	83
	Demeton+DDT	8.5	12.8	53	84
	Malathion+DDT	7.8	11.5	43	69
	DDT	7.8	11.3	30	65
Karathane	Lindane+DDT	8.2	11.9	43	82
	Demeton+DDT	8.3	11.8	48	77
	Malathion+DDT	8.0	11.4	42	70
	DDT	8.1	11.9	43	79
None	Lindane+DDT	7.5	10.8	29	60
	Demeton+DDT	8.4	12.1	52	77
	Malathion+DDT	8.2	11.4	51	71
None	None	6.8	9.9	8	34

[a] In sixteenths of an inch.

Table 5.—The effect of four annual biweekly lindane-sulfur-DDT sprays from 1956 to 1958 on the mean caliper (trunk diameter) and mean grade (caliper and height) of four varieties of 2-year old apple nursery trees at time of harvest, 1958.

Variety	Mean Caliper[a]	Trees in Grades			Per Cent Increase Over Unsprayed Control in
		1 or above	2	3	Mean Caliper
	Lindane-sulfur-DDT spray				
McIntosh	14.1	99	1	0	32
Idared	12.9	91	8	1	20
Golden Delicious	13.9	94	5	1	43
Monroe	13.2	99	1	0	55
	Unsprayed control				
McIntosh	10.7	55	35	10	—
Idared	10.8	58	33	9	—
Golden Delicious	9.7	24	52	23	—
Monroe	8.5	2	49	49	—

[a] In sixteenths of an inch on 2-year old trees.

Effect of Treatment on Variety.—The four apple varieties included in the experiment showed very pronounced differences in response to spraying (table 5). McIntosh and Idared, both varieties of innate vigorous growth habit in the nursery, showed the least response to the 3 years of spray applications, although they were benefited considerably. Golden Delicious, of medium vigor, and Monroe, of comparatively low vigor, demonstrated greater responses to spraying. Monroe, an extremely mildew-susceptible variety, showed a pronounced tendency to "feather," *i.e.*, to produce numerous secondary terminals from normally dormant axillary buds, when growth of the principal terminal was checked for any reason. "Feathering," since it results in a very bushy growth habit, is highly objectionable in a nursery tree; thus adequate control of potato leafhopper and of powdery mildew benefited Monroe more than any other varieties in the test (table 5).

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Insecticide Residues on Forage under Sprayed Pecan Trees[1]

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Abstract

Guthion® (*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3-(4*H*)-ylmethyl) phosphorodithioate), EPN and parathion applied to pecan trees in three applications at 2-week intervals using 2 pounds of wettable powder (15% or 25% active ingredient) 40% toxaphene in two applications 2 weeks apart using 6 pounds of wettable powder per 100 gallons of spray yielded initial deposits of forage under the trees of from approximately 1 to 60 p.p.m. for Guthion, 24 to 76 p.p.m. for EPN, 4 to 28 p.p.m. for parathion and 488 to 672 p.p.m. for toxaphene. Two weeks after any application, parathion residues were all below 1 p.p.m. with one exception during a dry period, Guthion residues ranged from 0.6 to 10.3 p.p.m. and EPN residues from 0.4 to 5.3 p.p.m. Toxaphene residues ranged from 69 to 126 p.p.m. after weathering 2 weeks and 23 p.p.m. remained 10 weeks after the second application.

The grazing of livestock in pecan orchards has become quite common in Georgia during the past 10 years. It is desirable, however, to treat pecan trees at times for insect control. This has created a need for information on the accumulation and fate of toxic residues on forage in sprayed orchards. Practically, livestock-pecan growers want to know how soon after spraying livestock may be allowed to graze in treated orchards. Although much work has been done on the determination of residues on forage treated uniformly with low dosages of insecticides, none has come to our attention concerning the accumulation of residues on forage in heavily sprayed orchards. Ordinarily, insecticides are used in much larger quantities per acre for controlling insects in orchards than on forage crops. Considerable spray-drip or runoff and some drift usually occurs when large trees are being thoroughly sprayed. The accumulation of much heavier insecticide deposits is to be expected, therefore, on forage under the tree than in the remainder of the orchard, or on forage treated specifically with a uniform application at the usually recommended rates.

The need for information as to when it is safe to allow livestock to graze in sprayed pecan orchards prompted experiments at Albany, Georgia, during 1957, 1958, and 1959 to determine the extent to which insecticide residues accumulate and persist on forage beneath sprayed trees. Several insecticides known to be useful in pecan insect control were included in the study.

MATERIALS AND METHODS.—A 15-acre block of pecan trees with primarily Bermuda grass ground cover was obtained for the experiments. In 1957 and again in 1958 wettable powers of parathion, EPN and Guthion® (*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3-(4*H*)-ylmethyl) phosphorodithioate) were each applied three times at 2-week intervals and in 1959 a toxaphene wettable powder formulation was applied twice 2 weeks apart to groups of four trees. All the applications were made with a hydraulic sprayer in the conventional manner, with about 40 gallons of spray per tree. A record was kept of the rainfall in the experimental plot throughout the work.

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[3] Chemist.

The grass samples for residue analyses were cut with a small power-driven sickle bar mower. Each year, before the initial samples were taken, the circular areas under the spread of the branches of the trees were separated roughly into the number of segmented areas of equal size needed for samplings during the season. There were nine segmented areas under each tree in 1957 and 1958, and 10 in 1959. In each experiment, immediately after the spray from the first application had dried, one segment of grass was cut from beneath each of four unsprayed trees and from under each sprayed tree. The first cutting under the first tree in each set was started at the tree skirt on the north side; from under the second tree on the east side; from under the third tree on the south side, and from under the fourth tree on the west side. Cuttings for subsequent samples were started where the last cuttings were terminated. A 5-pound composite sample of grass, $1\frac{1}{4}$ pounds from a segment beneath each of the four trees in a set, was collected for each treatment on each sampling date.

The samples were delivered in paper bags to the chemical laboratory at Tifton, Georgia, the day they were taken and processed promptly to prevent loss of insecticide prior to analysis. Upon receipt at the laboratory, the samples were reweighed, their weights were recorded, and then the forage was chopped into about 3-inch lengths with a modified motor-driven lawn mower and replaced in its original paper bag. Chopping produced mixing within the samples. Each sample in turn was then spread uniformly over a clean sheet of paper and a representative segment, including coarse and fine material, selected and weighed, recorded weights usually ranging from 200 to 300 grams. These samples were placed in clean, 1-gallon, friction lid buckets, 500 ml. of appropriate solvent was added, and extraction of the residue was continued for 1 hour on a stripping machine to remove surface residues from the plant material. An aliquot of the organic solution was filtered off from the grass for determination of insecticide present.

Guthion in the hexane extract was determined according to the procedure proposed by Meagher (1960), EPN and parathion in benzene extract according to the established procedure of Averell & Norris (1948), and toxaphene in hexane extract by the total chlorine method described by Phillips & DeBenedictis (1954), with improvements by H. W. Ruck (personal communication), Pesticide Chemicals Research Branch, Vincennes, Indiana.

RESULTS.—The insecticides, the number and time of applications, and the residues, expressed in parts per million on a green weight basis, found in samples after various periods of time are presented in tables 1 and 2.

In 1957 the residues found immediately following each of the three applications ranged from about 10 to 31 p.p.m. for Guthion, 24 to 36 p.p.m. for EPN, and from 10 to 26 p.p.m. for parathion. Residues declined rapidly during the succeeding 7 days and all were below 1 p.p.m. after 13 days, except following the second application of EPN, when 2 p.p.m. of that insecticide remained.

In 1958 initial deposits of Guthion and EPN were much larger than in 1957 and those of parathion were slightly higher following the first two applications, but somewhat lower following the third application. Part of the larger Guthion deposits can be attributed to the use of a 25% formulation in 1958, whereas a 15% material was used in 1957. As in 1957, residues declined rapidly during the succeeding 7 days. After 13 days residues ranged from about 0.8 to 10 p.p.m. for Guthion, 2.5 to 5.3 p.p.m. for EPN, and from 0.8 to 1.4 p.p.m. for parathion, or somewhat above the level of the year before. This fact may be explained in part by larger initial deposits in 1958 than in 1957 and in part by differences in rainfall. In 1957, 6.15 inches of rain fell during the course of the experiment, 4.04 inches of which occurred between the 7th and 13th days following the third application. During the 1958 experiment a total of only 3.22 inches was recorded, 2.99 of which occurred between the first and second applications. Only 0.23 inches fell during the entire period after the second application.

In the 1959 work with toxaphene, the residue found immediately after the first application was very heavy, analyzing 672 p.p.m. About two-thirds of this disappeared in 7 days and about 90% in 13 days, when 69 p.p.m. remained. Heavy deposits were found again immediately following the second application and from then on a slow

Table 1.—Summary of residue determinations on forage under sprayed pecan trees, Albany, Georgia, 1957–58.

Insecticide[b]	Days Following Treatment	Residues (p.p.m.)[a]					
		First Application		Second Application		Third Application	
		1957 (July 31)	1958 (July 29)	1957 (Aug. 14)	1958 (Aug. 12)	1957 (Aug. 28)	1958 (Aug. 26)
Guthion WP 15%[c]	0	10.2	22.1	15.0	61.6	31.5	40.2
	7	0.7	1.2	0.5	3.7	6.1	8.9
	13	0.6	.8	0.6	2.1	0.6	10.3
EPN WP 25%	0	36.4	76.4	33.2	39.0	24.5	33.2
	7	4.0	4.9	6.4	7.9	0.6	8.8
	13	0.9	3.5	2.0	2.5	0.4	5.3
Parathion WP 15%	0	25.6	27.6	10.1	16.9	10.8	3.7
	7	1.9	1.1	1.5	2.6	0.8	.2
	13	0.4	.8	0.4	.7	0.2	1.4

[a] Residues figures are means from duplicate analyses.
[b] Applied at 2 pounds of formulation per 100 gallons of water.
[c] 25% material used in 1958.

Table 2.—Summary of toxaphene residues on forage under sprayed pecan trees, Albany, Georgia, 1959.

DAYS FOLLOWING APPLICATION OF 6 LBS. 40% TOXAPHENE WP PER 100 GALS. WATER	RESIDUE (P.P.M.)[a]
First application (July 1)	
0	672
7	187
13	69
Second application (July 15)	
0	488
7	225
13	126
28	93
42	65
56	41
70	23

[a] Residues figures are mean from duplicate analyses.

disappearance took place, 23 p.p.m. remaining after 70 days. This slow decline took place even though 9.35 inches of rain fell during the experiment, 6.75 inches within the first 28 days.

The results show that, as used, parathion accumulated and persisted to a lesser extent than either Guthion or EPN. Guthion and EPN were about equally persistent; however, residues of both materials generally decreased by well over 90% in 13 days. The indications are that the amount of rainfall was an important factor in the persistence of residues of the insecticides used in these tests. Generally, it would seem that parathion residues on forage under the trees, following use of that insecticide for pecan insect control, would not exceed 1 p.p.m. after 13 days weathering, except possibly in extremely dry periods. This is a level which, it is understood, will not contribute residues to meat or milk. On the other hand, since the safe level for Guthion or EPN on grass forage has not been determined, livestock should not be pastured in orchards in which those insecticides are used so long as it is suspected that any residue resulting from their use may remain on the forage.

Toxaphene residues were very high and more persistent than those of the phosphorus insecticides tested. The extrapolated data, based on the residue found at intervals during a 70-day period following the second application, indicate that it would probably be well over 100 days before the residue would decline to 7 p.p.m., the tolerance for toxaphene on many crops. Although there is approval for grazing meat animals to within 6 weeks of slaughter in pastures treated with not over 1½ pounds toxaphene per acre per season, there remains to be determined, as with Guthion and EPN, the waiting period after which such animals can be allowed to graze in orchards sprayed with that insecticide in the amount needed for controlling pecan insects.

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Observations on the Desert Grasshopper, *Trimerotropis pallidipennis pallidipennis*, in Arizona[1]

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ABSTRACT

Between 1936 and 1959 this was one of the most widely distributed grasshopper species in Arizona, both as to location and type of habitat. Populations averaged higher at moderate than at very low or very high elevations. The highest and most extensive infestations occurred in 1941 and 1958, mainly on nonirrigated desert land, in south-central Arizona following continuous fall-winter-spring periods when precipitation and the condition of desert vegetation were much above normal. *Trimerotropis pallidipennis* often migrated into cultivated land and caused damage to crops. Severe damage to seedling cotton in Pinal and Maricopa Counties occurred in 1958. It has a spring generation throughout its range in Arizona, and usually at least one additional generation at elevations below 5,000 feet. Desert populations were reduced 74% in 1 day and 86% in 6 to 7 days by an aldrin spray applied by aircraft in the spring of 1958.

The heaviest infestation of *Trimerotropis pallidipennis pallidipennis* (Burm.) in 24 years developed in the spring of 1958 in south-central Arizona. Highest populations were observed in Maricopa and Pinal Countries. Hatching of the first, or spring, generation was mainly on desert land. Substantial damage to crops in adjacent cultivated areas required control. Field observations on this grasshopper during the 1958 outbreak and other information obtained in study areas during the period 1936–57 are presented herein.

OBSERVATIONS IN 1936–57.—Ball *et al.* (1942) reported *pallidipennis* as one of the most abundant grasshoppers in the Sonoran zones, up to 8,500 feet elevation. Surveys by U. S. Department of Agriculture workers between 1936 and 1957 showed it to be one of the most widely distributed grasshoppers in Arizona. It was taken from desert land with an annual precipitation of 3 to 4 inches and an elevation of 100 feet to forest rangeland with an annual precipitation of 18 inches and an elevation of over 7,000 feet. There were great fluctuations in populations

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[2] In cooperation with the Arizona Agricultural Experiment Station.

Table 1.—Populations of *Trimerotropis pallidipennis pallidipennis* adults in four areas in Arizona, 1937–44.

Area	Mean Temperature (° F.)	Mean Annual Precipitation (Inches)	Elevation (Feet)	Number per Square Yard	Per Cent of Total Population
South Gila Valley (near Yuma)	73.2	3.53	150	0.020	2.8
Salt River Valley (near Tempe)	67.9	8.50	1,100	.072	15.8
Chino Valley[a]	56.4	13.02	4,600	.100	10.0
Black Bill Park (near Flagstaff)	46.7	16.46	6,800	.013	5.5

[a] Records for 1937–43 only.

Table 2.—Adult populations of *Trimerotropis pallidipennis pallidipennis* in the Salt River Valley near Tempe, Arizona, 1937–44.

Year	Mean Temperature (° F.)	Annual Precipitation (Inches)	No. per Square Yard	Per Cent of Total Population
1937	67.9	7.42	0.090	17.3
1938	67.7	5.63	.023	5.5
1939	68.3	6.34	.069	16.4
1940	69.4	9.00	.031	11.1
1941	67.0	18.08	.338	54.5
1942	67.5	4.85	.034	8.7
1943	69.0	8.15	.007	4.4
1944	66.8	8.51	.042	5.0
Average	67.9	8.50	.072	15.8

Table 3.—*Trimerotropis pallidipennis pallidipennis* populations and the percentage of *pallidipennis* in grasshopper collections in alfalfa, Salt River Valley area near Tempe, Arizona, 1936–45.

Year	Average No. per Square Yard	Per Cent of Total Population
1936	0.081	3.5
1937	.007	.5
1938	.010	.4
1939	.132	14.6
1940	.045	5.3
1941	.360	26.5
1942	.062	5.3
1943	.009	1.6
1944	.048	2.1
1945	.191	2.6
Average	.094	6.2

between years, areas, and plant habitats. It was usually most noticeable in open, dry areas, but in dry periods it concentrated in low areas or along washes in non-irrigated land, sometimes moving to irrigated cropland.

Table 1 shows average adult populations for the 1937–44 period in four 6- to 10-section blocks of cultivated and uncultivated land in four widely separated areas with great climatic differences. Populations were greater and comprised a higher percentage of the total grasshopper population in areas where temperature, precipitation, and elevation were intermediate.

Table 2 shows populations for the same period in a 10-section area of the Salt River Valley near Tempe. The highest population as well as its high percentage in the total grasshopper population occurred in 1941, a year with lower than average temperature and about twice the average rainfall.

Table 3 shows *pallidipennis* populations and the percentage of this species in grasshopper collections in alfalfa in the Salt River Valley area for the period 1936–45. The outstanding features were the unusually high population and the high percentage of this species in 1941, the year with the highest rainfall.

In 1941 *pallidipennis* was clearly dominant, comprising 56% to 94% of the grasshopper population in 10 of 13 plant associations studied and 20% to 29% in the other three. There were more of this species in 1941 than of any other observed in the area since the surveys were begun. There was slight damage to young cotton in some fields and 12% damage to carrot plants in one field that bordered on weedy river-bottom land. The adult population in the carrot field reached seven per square yard.

The upsurge in the summer of 1941 was caused by unusually favorable conditions during the preceding fall, winter, and spring. Rainfall excesses of 0.36, 1.25, and 3.33 inches in September, October, and December 1940, respectively, assured plenty of succulent food for ovipositing females. Winter temperatures averaged 2.5° F. above normal, perhaps increasing the life span and egg production of females. The spring-summer period (March-August) with an average daily temperature deficiency of 3.2° F. and a rainfall excess of 6.87 inches, was favorable to plant growth in nonirrigated and desert land. Plants were green and succulent longer than usual, providing ample food and more than the normal amount of shade and shelter for the nymphs and adults. Above-normal soil moisture was probably conducive to egg survival and high hatching percentages.

The percentage of *pallidipennis* remaining on the desert through the summer of 1941 was higher than usual, but notwithstanding this the number of grasshoppers that migrated into cultivated land was larger than in any other year during the period 1936–45. Spring nymphal populations exceeded 50 per square yard in limited areas of desert and river-bottom land near Tempe and Gila Bend, both in south-central Arizona.

Referring to *pallidipennis*, Ball *et al.* (1942) wrote: "Breeds in areas of thin soil and sparse vegetation. Migrations occur especially in June and July at which time much damage may be done to cultivated crops. Many crops are injured, the most important being vegetables, alfalfa, milo, barley, cotton, and corn. Large numbers may come to lights and be very annoying. . . ."

Aside from the 1941 outbreak, migrations of this species into cities and cultivated areas in south-central and other sections of Arizona were observed at Flagstaff, Ash Fork, and Prescott and southward to Willcox, Tucson, Phoenix, and Yuma.

In central and southeastern Arizona *pallidipennis*, often in association with *Conozoa sulcifrons sulcifrons* (Scudder) or *C. carinata* Rehn, caused injury to vegetable crops, particularly young plants near desert or rangeland on which the vegetation was drying up or dry. For example, in the fall of 1947 near Tempe adults in moderate numbers came into a small field bare of vegetation except for young beet plants and damaged or completely destroyed many of the small seedlings in a short time. Immediate control was necessary and a poisoned bait was found effective.

Lauderdale (1950) noted that in 1949 "Fall vegetables suffered from migrations of . . . and the desert grasshopper

(*Tremerotropis pallidipennis*). Many fields had to be replanted as a result of the depredations of these pests." It is probable that he was writing of fields in south-central Arizona.

Some of the largest migrations occurred in 1954. In late September and early October adults migrated from desert and rangeland to lighted street areas in Phoenix, Mesa, Globe, Safford, and Willcox. At Globe it was necessary to close the streets to traffic while firemen washed away the remains of the crushed grasshoppers. Normal to above-normal rainfall and plant growth on desert and rangeland during the summer of 1954 in many areas of southern Arizona evidently favored the increase of this grasshopper.

In the spring of 1955 a threatening to heavy infestation developed on about 2,000 acres of desert land northwest of Scottsdale. In mid-April most of the grasshoppers were in the nymphal stage. Green to drying, sparse to dense vegetation was 1 to 6 inches high and consisted mainly of alfilaria with some Indian wheat, peppergrass, bur-sage, and *Cryptantha* sp. The nymphs fed to some extent on all the plants. Populations ranged from 1 to 100 or more per square yard and were highest in low areas or along washes where the vegetation was most dense and succulent. There had been no migration into adjoining plowed land to the south where cotton was being planted. Later, as the nymphs developed and the cotton came up, many moved into the fields, fed on the young plants and had to be controlled with insecticides.

THE 1958 OUTBREAK.—In 1958 *pallidipennis* populations were above normal in most of Arizona. Surveys in April and early May disclosed spotty to extensive economic infestations of five or more per square yard on desert land in Yavapai, Maricopa, and Pinal Counties. The highest populations, 50 to 100 or more per square yard, were seen late in April in Maricopa and Pinal Counties.

The nymphs preferred weedy plants (forbs), but also fed considerably on quick-growing annual grasses. Among the preferred plants were alfilaria, globemallow, peppergrass, and deer-vetch. They also fed on Indian wheat, bur-sage, and other plants. Populations were highest in fairly dense stands of mixed weedy vegetation in depressions and washes.

During the last half of April and the early part of May as the desert vegetation dried, many late-instar nymphs of the first generation moved into nearby fields where the vegetation was more succulent. Many adults also flew to cultivated areas or to rangeland at higher elevations. In some desert areas, particularly near Apache Junction and Florence Junction, there was high nymphal mortality apparently caused by scarcity of suitable food.

Although *pallidipennis* did much damage to desert-type range vegetation, the greatest economic injury was caused by migrating nymphs and adults that fed on crops, including cotton, alfalfa, small grain, and vegetables. Marginal to severe damage to seedling cotton was observed from Harquahala Plains and Wintersburg to Litchfield Park, Tempe, Scottsdale, and southeastward to Casa Grande and Florence. The most severe damage occurred in the Maricopa-Casa Grande-Florence area of Pinal County, where it was necessary to replant large acreages once or twice to get a good stand. The grasshoppers chewed off the stems of young plants near the ground. In some fields of small grain, especially those near the desert in the Casa Grande and Litchfield Park-Perryville areas, the plants were defoliated and some of the heads cut off. Damage to alfalfa was less than expected from the populations seen in alfalfa fields. Populations in cotton were usually 5 to 10 per square yard or lower, but in some fields of alfalfa and small grain they reached 25 to 50. Damage to crops was most noticeable in fields near extensive areas of desert.

Hordes of migrating adults were a nuisance for several days in early May in Phoenix, Casa Grande, Tucson, and other cities, but they caused little damage to vegetation. The flying adults interfered slightly with air traffic. Pilots reported encounters with the adults at elevations of 3,000 to 5,000 feet.

By early June the population in south-central Arizona had declined to noneconomic status in nearly all areas and habitats. The decrease was due to dispersals, migration, and mortality from natural causes and insecticide applications. During the early part of the summer, populations were very low in all low-elevation, nonirrigated habitats. In August and September after rains and the reappearance of green vegetation on the desert, adults including some gravid females, were concentrating, up to 2 to 5 per square yard, in small low areas or along washes where the vegetation was tallest and most succulent and dense. On the average, however, late-summer and fall populations on bare to sparsely vegetated, uncultivated, or nonirrigated land were below 0.5 per square yard.

The outbreak of *pallidipennis* in the spring of 1958 was caused by above-normal and well-distributed rainfall during the preceding fall, winter, and early-spring period, which favored soil and plant conditions that were favorable to the grasshopper. Based on Weather Bureau records, rainfall for the South-Central Division of Arizona averaged 11.3 inches, or about 1 inch above normal, for the 12-month period ending April 30. Although the excess in rainfall was not great, it was almost perfectly distributed to increase the fall and spring growths of desert plants and lengthen their periods of succulence. An adequate supply of succulent food over a long period, together with moist soil conditions suitable for egg laying, preservation, and hatching, favored the development, survival, and increase of *pallidipennis*. The stand and growth of desert vegetation were much above normal in the fall of 1957 and the spring of 1958.

Rainfall for 17 stations showed average excesses of 2.62 inches in October 1957 and 0.39, 1.05, and 0.43 inches, respectively, for February, March, and April 1958. The October rainfall was about 657% of normal and that for March and April 202%. The fall-winter-spring period for growth of desert vegetation started earlier and continued until later than normal.

In representative alfalfa fields in the Salt River Valley, the average grasshopper population, 0.535 per square yard, and the percentage of *pallidipennis*, 73.4, were substantially higher than the 0.36 per square yard and 26.5% in 1941.

SEASONAL DEVELOPMENT.—In 1958 in south-central Arizona, hatching of the first generation occurred mostly in late February and during March. By mid-April late-instar nymphs comprised more than 99% of the popula-

tion. By the last of April adults were greatly dominant but late-instar nymphs were still present. Females with well-developed eggs were seen in succulent vegetation in irrigated fields and margins as early as the first week in May, but none in dry desert habitats up to the middle of June. Similar females were observed on the desert in September after a new growth of grass and weeds had come up following summer rains. They were most numerous along roadsides and washes where the vegetation was most succulent and dense. Prominent plants in preferred habitats were spurge, fluffgrass, and puncture vine, all of which were fed upon. In some irrigated and marginal habitats, as alfalfa and weedy field margins, early-instar nymphs of the second generation were taken in collections made June 5 to 18. Light populations of second-generation nymphs caused slight damage to young cotton near margins in a few fields adjacent to desert and reverted land near Casa Grande during June.

Periodic observations between 1936 and 1959 have shown that *pallidipennis* may often have more than one generation a year at elevations below 5,000 feet. There is a spring generation usually followed by one or more generations or partial generations during the summer and fall. Second or later generations may not occur in non-irrigated habitats when there is very little summer rain. Hatching of the first generation comes later in the cooler, higher areas. That of the second generation depends more on suitable food for the first generation and ample spring and summer soil moisture than on temperature. Locations where two or more generations a year have been observed are Chino Valley, Cottonwood, Congress Junction, Peeples Valley and Skull Valley in Yavapai County, San Carlos Apache Indian Reservation, Bonita and Sunset in Graham County, Aguila, Buckeye Valley, Salt River Valley, Gila Bend and Wickenburg in Maricopa County, Apache Junction, Bon, Casa Grande, Florence Junction and Maricopa in Pinal County, and South Gila Valley and Yuma Valley in Yuma County. Only a single generation has been observed at Black Bill Park, Flagstaff, and Fort Valley, all in Coconino County at elevations of 6,800 to 7,300 feet with very short growing seasons.

In addition to 1958 field observations, evidence of more than one generation of *pallidipennis* was obtained in laboratory cages at Tempe. Late-instar nymphs of the first generation were collected on April 16 from desert land near Florence Junction, placed in a screen cage, and brought to the laboratory. They were held at ordinary room temperature and furnished a mixed diet of succulent alfalfa, Johnson grass, and head lettuce. After the nymphs became adults, pots of moist sand were put in the cages. to receive eggs that might be laid. Several pods of eggs that were laid on June 6 were placed over moist sand at room temperature and examined frequently. On July 5 many first-instar nymphs had hatched, others were hatching, and most of the remaining eggs had embryos up to the hatching stage. Embryonic development and hatching occurred without subjection of the eggs to low temperatures.

CONTROL.—In tests and observations in alfalfa and on rangeland during the period 1937–53, *pallidipennis*, along with most other grasshopper species, was susceptible to various insecticides (arsenicals, sodium fluosilicate, toxaphene, chlordane, aldrin, dieldrin, heptachlor, parathion, etc.) applied in sprays, dusts, or baits. However, attempts to control the migrating nymphs and adults in seedling cotton in the spring of 1958 were only partially successful. Sprays applied to fields and margins killed many grasshoppers, but others continued to come in from desert land, cutting off many slender seedlings before swallowing enough insecticide to cause death. It became evident that control on desert land would be necessary to prevent migration into nearby fields.

In May a cooperative control program was carried out by the Arizona Commission of Agriculture and Horticulture, the United States Department of Agriculture, and farmers concerned on approximately 138,000 acres of desert land in the Maricopa-Casa Grande-Florence area and in some of the smaller infested areas in Pinal County and the extreme southeastern part of Maricopa County. Two ounces of aldrin in 1 gallon of diesel fuel was sprayed on each acre. Application was by aircraft between 6:15 and 8:00 A.M. on May 5 and 6 on strips about 2 miles wide adjacent to cultivated areas.

At the request of the control agencies, on May 5 to 12 the author checked grasshopper reductions from the treatment at six desert locations $3\frac{3}{4}$ miles northwest to $13\frac{1}{2}$ miles northeast of Casa Grande. Nearly all grasshoppers were adult *pallidipennis*. Counts were made on 20 one-square-yard areas 20 steps apart at each location. The terrain was level to gently sloping with many shallow washes. Vegetation, mostly sparse but with scattered dense spots, consisted of dry or drying annual grasses, Indian wheat, peppergrass, and *Cryptantha* sp. Sparse to moderate stands of shrubs at some locations consisted of green to drying saltbush, bur-sage, creosotebush, and mesquite. Populations at time of spraying ranged from 1.7 to 3.85 per square yard and averaged 2.4. Average reductions for the six locations 1, 3 to 4, and 6 to 7 days after treatment were 74%, 85%, and 86%, respectively. Reductions were fairly uniform at the different locations, ranging from 82% to 91% after 6 to 7 days.

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Effect of Topical Applications of Granulated Systemic Insecticides and of Conventional Applications of Other Insecticides on Control of Insects and Spider Mites on Sugar Beet Plants[1]

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Abstract

Granulated phorate and granulated *O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorodithioate (Di-Syston®) were applied topically to large sugar beet plants. With this method of application the large leaves and flat petioles funneled most of the granules onto the plant crown. Both materials gave excellent and persistent reductions of the green peach aphid, *Myzus persicae* (Sulz.), the southern garden leafhopper, *Empoasca solana* DeL., and the spider mite, *Tetranychus cinnabarinus* Bvd., the three most important pests on sugar beet plants in the Imperial Valley after the seedling stage of growth.

No residues of either phorate or Di-Syston were detected in the large roots, but phorate residues in the foliage were 0.07 p.p.m. 29 days after application.

Of other materials evaluated, Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide) gave excellent mortality of the green peach aphid; Sevin® (1-naphthyl methyl-carbamate) and ethion were particularly promising when used for control of the southern garden leafhopper; and ethion, Trithion® (*S*-(*p*-chlorophenylthiomethyl)*O,O*-diethyl phosphorodithioate), demeton, and Kelthane® (4,4′-dichloro-*alpha*-(trichloromethyl)-benzhydrol) were all effective in reducing populations of spider mites.

Sugar beet production in the Imperial Valley of California is somewhat unique in that planting takes place primarily during September and October; harvest follows in late spring and early summer and is completed by the end of July. By way of contrast, in most areas sugar beets are planted in the spring of the year and are harvested in the fall.

The fall planting of sugar beets in the Imperial Valley presents a problem the following spring when it is sometimes necessary to control heavy infestations of insects and spider mites with chemicals. The problem in control arises from the fact that the foliage of the sugar beet plant is very dense at this time, the leaves being about 2 or more feet long and nearly completely filling the irrigation furrows. The problem then, particularly with aphids and spider mites, is primarily one of plant coverage with the chemicals, coverage of the lower leaf surface being especially important in the case of spider mites, and penetration to the small leaves at the plant crown, in the case of aphids. That plant coverage with conventional chemicals is difficult to attain on plants as large as these is illustrated by results shown in the following tables.

Although other pests are found, in the spring of the year three species predominate and reach damaging population levels in many fields. The green peach aphid, *Myzus persicae* (Sulz.), beginning usually in December or January, infests practically all fields, the severity and persistence of the infestation varying from year to year and depending largely upon the numbers of beneficial insects present, aphid disease, or the advent of hot weather. In most years the aphid infestation is highest in February and declines drastically in March. At about this time a few spider mites, *Tetranychus cinnabarinus* Bvd., and the southern garden leafhopper, *Empoasca solana* DeL., are becoming evident in the field. In the latter part of April and in May, the two last-named species reach such large numbers in some fields that chemical treatment is required. Eventually, the spider mite population may be decimated, primarily by the activities of six-spotted thrips, *Scolothrips sexmaculatus* (Perg.), and *Orius* sp., but in the meantime serious plant damage may take place.

Materials and Methods.—A number of materials were applied in these tests. All except the granulated systemic chemicals were applied in a conventional manner as either sprays or dusts. Details are given in the following description of experiments.

Most research with granulated systemic insecticides has made use of the ability of these chemicals to absorb into and translocate from the roots into the aerial portions of the plant following application to the soil. As it is difficult to side-dress or place a band of granules in the soil alongside the sugar beet row when the roots are large, the applications were topical. In this type of application the granules are allowed to flow gently along and over the plant row. The large leaves and flat petioles act somewhat as funnels, so that a large part of the material tumbles onto the plant crown, although some granules remain on the leaves or fall to the ground.

In small plots, applications were made with a hand-duster operated with a minimum of blast or air velocity. In larger plots, applications were made with fertilizer attachments mounted on a tractor, four fertilizer application units being mounted so as to treat four rows simultaneously. After careful calibration of the fertilizer units, the flexible tube outlets were arranged so that the granules flowed over the center of the sugar beet row. All granulated applications were made when the plants were dry.

In each experiment the treatments were replicated four times. The replicates or plots in the various experiments varied in size from one row wide and 50 feet long to as much as 60 rows wide and one-fourth mile long.

Field Results.—*Experiment No. 1*—Treatments for spider mite control were made on May 7, 1957, on a field of nearly mature sugar beets near El Centro. In this particular field there were two rows of sugar beets in each bed. All applications were made with hand equipment. Sprays were applied with a hand-compression sprayer equipped with a hollow-cone nozzle and operated at a

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Table 1.—Results of dust and spray applications of conventional acaricides and topical applications of granulated phorate and Di-Syston made May 7 on large sugar beet plants for control of *Tetranychus cinnabarinus* Bvd. in the Imperial Valley, 1957.

Material and Formulation	Amount Applied per Acre	Toxicant per Acre (Lb.)	Number of Spider Mites Counted for 60 Leaves					
			May 14		May 21[a]		May 28	
			Adult	Immature	Adult	Immature	Adult	Immature
Phorate, 2% granule	55 lbs.	1.1	5	0	141	4	45	24
Di-Syston, 2% granule	80 lbs.	1.6	108	94	132	28	31	28
Ethion, spray	26 gals.	1.3	7	1	186	5	73	46
Demeton, spray	28 gals.	0.7	42	50	234	33	112	88
Delnav, spray	24 gals.	0.8	100	176	247	175	211	276
Kelthane, 3% dust	50 lbs.	1.5	16	119	262	46	112	127
Trithion, 3% dust	40 lbs.	1.2	33	38	515	93	204	177
Thiodan, 3% dust	40 lbs.	1.2	123	102	236	165	228	273
Check	—	—	279	305	445	438	315	574

[a] A strong wind between May 14 and May 21 apparently blew many adult mites into the plots.

pressure of 40 p.s.i. Both dusts and granules were applied with a rotary hand-duster to plots, two sugar beet beds (4 rows) wide and 50 feet long. Treatments were evaluated 7, 14, and 21 days after application. Sixty newly mature leaves (15 per replicate) were collected from plants of each treatment and transported in iceboxes to the laboratory for spider mite counts. Because of the high mite populations, circles seven-eighths inch in diameter were stamped on the lower surfaces of the leaves, and adult and immature mites within the marked areas were counted. The materials used, rates of application, and results obtained are shown in table 1.

As shown in table 1, most of the materials gave good spider mite reductions within 7 days. At the dosages used, demeton and ethion sprays, Kelthane® (4,4′-dichloro-*alpha*-(trichloromethyl)-benzhydrol) and Trithion® (*S*,(*p*-chlorophenylthiomethyl)*O*,*O*-diethyl phosphorodithioate) dusts, and phorate granules gave good reductions. Applications of Di-Syston® (*O*,*O*-diethyl *S*-2-(ethylthio)ethyl phosphorodithioate) granules were characteristically slow in reducing the population, but eventual reductions were excellent. Neither Delnav® (2,3-*p*-dioxanedithiol *S*,*S*-bis(*O*,*O*-diethyl phosphorodithioate) nor Thiodan® (6,7,8,9,10,10 - hexachloro - 1,5,5*a*,6,9,9*a* - hexahydro - 6, 9-methano-2,4,3-benzodioxathiepin-3-oxide) gave good mite reductions. A strong wind between May 14 and May 21 apparently increased the mite population in the plots, possibly by blowing in adults from the surrounding planting. As a consequence, the adult population was much higher on May 21, the Trithion-treated plots being particularly affected. At the time of the final count the plots treated with ethion, phorate, and Di-Syston had the lowest mite populations, with demeton and Kelthane-treated plots nearly as good.

Experiment No. 2.—On June 25, 1957, granulated demeton and Di-Syston were applied to maturing sugar beet plants near Calexico for control of the southern garden leafhopper. In this field there was a single row of plants in each bed. The granules were applied topically with a rotary hand-duster, the materials being directed over the top of the plant row so that the granules tumbled into the plant crowns at the base of the leaves. Each plot was 1 row wide and 50 feet long. Leafhopper mortalities were evaluated on July 2 and 10. Leafhopper nymphs were counted on a total of 40 leaves per treatment (10 per replicate). As the field was becoming somewhat dry for harvesting the beets, mature leaves showing good color were selected for evaluation. Only immature leafhoppers were counted, as mature leafhoppers could readily reinvade the small treated area. Rates of application and results obtained are shown in table 2.

The results presented in table 2 show that dosages of 6 ounces per acre of either phorate or Di-Syston were not adequate for satisfactory mortalities of the leafhopper nymphs. The 9-ounce dosage gave more satisfactory results. Since the dosage contemplated for use on this crop is 16 ounces per acre, either material should give excellent leafhopper control. As indicated in the previous experiment for control of spider mites, Di-Syston again was considerably slower than phorate in bringing about desirable reductions in the population.

Experiment No. 3.—On April 29, 1958, applications were made in a sugar-beet field near Calipatria, in which there was a heavy population of spider mites and a moderate population of leafhoppers. Applications were made with power equipment. Liquids were applied with a sprayer equipped with a 30-foot boom. Twenty hollow-cone nozzles were evenly spaced along the boom, and the sprays were applied at a pressure of 60 p.s.i. The granulated phorate was applied by means of tractor-mounted fertilizer attachments arranged to treat four rows simultaneously in the manner previously described. Each plot was 12 rows (30 feet) wide and 1,250 feet long. Twenty-five leaves were collected from each replicate (100 per treatment) in order to evaluate effectiveness of

Table 2.—Effects of granulated phorate and Di-Syston when applied to mature sugar beet plants for control of the immature *Empoasca solana* in the Imperial Valley, June 25, 1957.

Granulated Material Applied	Pounds per Acre	Toxicant per Acre (Oz.)	Leafhopper Nymphs Counted per 40 Leaves	
			July 2	July 10
Phorate, 2%	28	9	57	33
Phorate, 2%	19	6	154	124
Di-Syston, 2%	28	9	140	18
Di-Syston, 2%	19	6	228	159
Check	—	—	343	258

Table 3.—Comparative effectiveness of granulated phorate applied topically, and of several materials applied as sprays, for control of *Tetranychus cinnabarinus* and *Empoasca solana* on sugar beet plants in the Imperial Valley, April 29, 1958.

Material and Formulation	Amount Applied per Acre	Toxicant per Acre (Oz.)	Number of Spider Mites Counted per 100 Leaves						No. of Leafhoppers Counted per 80 Sweeps of Net		
			May 6		May 14		May 20				
			Adult	Immature	Adult	Immature	Adult	Immature	May 6	May 14	May 20
Phorate, 2% granule	42 lbs.	13.5	93	552	410	444	369	466	37	38	837[a]
Trithion, spray	9.2 gals.	11.0	142	1,214	698	1,148	—	—	238	250	—
Ethion, spray	8.8 gals.	10.5	412	2,178	1,068	1,699	—	—	120	57	245
Parathion, spray	9.4 gals.	7.5	1,020	2,989	949	2,233	—	—	72	83	371
Sevin, spray	10.0 gals.	16.0	1,016	2,432	789	2,317	—	—	27	25	228
Check	—	—	940	2,221	762	2,463	347	855	342	246	775

[a] As a result of good spider mite control, the phorate-treated plots remained green, and large numbers of adult leafhoppers were attracted to these areas.

treatment on the spider mite populations. Counts were made as described in Experiment No. 1. The effects of the various treatments on the leafhopper populations were evaluated by making 20 sweeps per replicate (80 per treatment) with an insect net. Counts were made in the field. The materials, rates of application, and results obtained are shown in table 3.

At the dosages used in this experiment, the granulated phorate was the only material that gave satisfactory control of both spider mites and leafhoppers. Both parathion and Sevin® (1-naphthyl methylcarbamate) gave good reductions of the leafhopper population, but neither one was effective for control of spider mites. Neither ethion nor Trithion, which at higher rates in Experiment No. 1 gave satisfactory results for mite control, was satisfactory at lower rates used in this test. Ethion gave promising leafhopper reductions, however. It should be noted that on May 20, the date of the last count, leafhopper populations were high in the phorate-treated plots. This undoubtedly was the result of good spider mite control. Plants in the phorate plots were much greener than those in the other treatments, and adult leafhoppers were attracted to these greener areas.

Experiment No. 4.—Treatments for control of green peach aphids were applied on January 23, 1958, in a field of sugar beets located near Calipatria. Applications were made with hand equipment, the sprays being applied with a hand-compression sprayer and the granules with a small hand-duster. The sprays and granules were applied in a manner similar to that described in Experiment No. 1. Each plot was one row wide and 50 feet long.

Treatments were evaluated in the field by counting the surviving aphids. Immature leaves located in the crown of the plant were selected for the counting. Eighty leaves (20 per replicate) were examined in each treatment. The materials used, rates of application, and results obtained are shown in table 4.

As in the previous experiments for spider mite and leafhopper control, the granulated systemic materials gave excellent reductions of the green peach aphid, particularly at the higher dosage. As expected from data obtained in previous work, phorate reduced the aphid populations more quickly than Di-Syston did, but eventual results were quite similar. The Thiodan spray and granules gave surprisingly good results, and residual activity, particularly in the case of the granules, was quite good. Results with endrin were only moderately good, and aphid mortalities obtained with demeton and parathion were poor.

Experiment No. 5.—On January 13, 1959, and again on March 3, applications for control of the green peach aphid were made on plants of a large field of sugar beets near Calipatria. In this instance the first treatment was made before the aphid population became abundant. The plots were relatively large, each granulated Thimet plot being 40 rows wide and each Thiodan spray plot 24 rows wide. Each of the check plots was 64 rows wide, and all plots extended the length of the field (1,200 feet).

The granulated phorate was applied with four fertilizer attachments mounted on a tractor as previously described. The Thiodan spray was applied with a power sprayer equipped with a 30-foot boom. The spray nozzles were arranged in such a way that one flat-fan nozzle was mounted directly over a sugar beet row. The sprays were applied at a pressure of 60 p.s.i.

Treatments were evaluated by counting apterous aphids on 25 leaves per replicate (a total of 100 leaves per treatment). Immature leaves in the crown of the plant were selected for aphid counts, as described in previous tests. Rates of application and results obtained are shown in table 5.

Both chemicals in this experiment gave excellent and persistent aphid control. Results with Thiodan were superior to those obtained with the same material in Experiment No. 4, probably because the aphid population was considerably less abundant and the residual period of toxic activity prevented aphid establishment from immigrating alate aphids. Results with Thimet were not as good as expected. This was apparently the result of a rising wind during the application on January 13. Only the last two of the four replicates treated with Thimet were adversely affected. It was noted that the wind deflected the granule flow from the crown of the sugar beet plant, and a considerable proportion of the granules was blown into the irrigation furrows. That effectiveness was somewhat reduced in the two affected replicates was revealed by the fact that practically all the aphids found

Table 4.—Comparative effectiveness of various insecticides and granulated phorate and Di-Syston for control of the green peach aphid on large sugar beet plants, January 23, 1958.

Material and Formulation	Amount Applied per Acre	Toxicant per Acre (Oz.)	Green Peach Aphids Counted per 80 Leaves		
			Jan. 27	Feb. 10	Feb. 21
Phorate, 2% granule	50 lbs.	16.0	11	42	114
Phorate, 2% granule	25 lbs.	8.0	18	225	296
Di-Syston, 2.5% granule	50 lbs.	16.0	296	94	112
Di-Syston, 2.5% granule	25 lbs.	8.0	341	554	282
Thiodan, 3% granule	33 lbs.	16.0	755	443	213
Thiodan, spray	25 gals.	20.0	593	853	315
Endrin, spray	20 gals.	8.0	883	1,446	881
Demeton, spray	22 gals.	4.4	1,175	2,294	—
Parathion, spray	24 gals.	9.6	1,409	3,736	—
Check	—	—	2,924	3,230	2,754
L.S.D. at 5% level			562	696	—

Table 5.—Effects of topical applications of granulated phorate, and of Thiodan applied as spray, for control of green peach aphid, spider mite, and leafhopper populations on sugar beet plants in the Imperial Valley, January 13 and March 3, 1959.

Material and Formulation	Toxicant per Acre (Oz.)		Number per 100-Leaf Sample on Date Counted								
			Green Peach Aphids							Spider Mites	Leafhopper Nymphs
	Jan. 13[a]	March 3	Jan. 22	Jan. 27	Feb. 3	Feb. 10	Feb. 17	Feb. 25	Mar. 19	May 7	May 7
Phorate, 5% granule	12	19	9	38	51	27	50	191	1	488	13
Thiodan, emulsion spray	14	17	11	10	1	2	5	33	8	3,268	143
Check	—	—	462	700	644	277	433	1,049	70	11,631	114

[a] During the application of phorate on January 13, a rising wind drastically affected the last two of the four replicates treated; a large percentage of the granules were blown into the irrigation furrows, and effectiveness was thus greatly reduced in the two replicates affected. Practically all the aphids found in the phorate-treated plots until after second treatment were in the two replicates receiving the poor application.

in the Thimet-treated plots were in the two replicates treated while high winds prevailed.

On May 7 it was observed that the field was becoming quite heavily infested with spider mites and leafhoppers. It was further noted that the Thimet-treated areas were considerably greener than the adjacent check and Thiodan-treated plots. A spider mite count revealed a light infestation in the Thimet plots only; whereas the untreated area was heavily infested. The Thiodan treatments had also inhibited mite buildup to quite an extent, even though this chemical is considered only a mildly effective acaricide. There was no apparent difference in adult leafhopper infestation, but a count of nymphs revealed a much lighter population in the Thimet plots than in the Thiodan plots and untreated areas, which were similarly infested.

Residue Studies.—The tops of the beet plants were removed, washed with 1 liter of water per kilogram of top, and then washed again thoroughly. The first liter of wash water was retained for analysis. Both the tops and the radial sections of the roots were chopped, 100-gm. subsamples were taken and homogenized with 200 ml. water in a Waring Blender. The homogenate was filtered through cheesecloth, and phorate and Di-Syston residues were determined by the electrometric cholinesterase procedure as described previously for alfalfa (Reynolds *et al.* 1957). The residues were calculated as *O,O*-diethyl *S*-2-(ethylsulfinyl)methyl phosphorothiolate (phorate oxygen analog sulfoxide) and *O,O*-diethyl *S*-2-(ethylsulfinyl)ethyl phosphorothiolate (demeton-thiol isomer sulfoxide) for Di-Syston. Results obtained are shown in table 6.

These data certainly show that no material is translocated into the root and thus that no residue problem exists in this important plant part. This is consistent with reports of systemics available to date, in that there is little or no movement of material down the plant from the point of application.

The major problem is one of residues persisting in the foliage. This is occasioned by the fact that following harvest, in the Imperial Valley, at least, beef animals are permitted to feed on the sugar beet tops. When the data on residues on sugar beet tops are projected as logarithmic plots to give straight-line residue persistence curves (Gunther & Blinn 1955), it is found that detectable residues are below the limits of test sensitivity (0.05 p.p.m. for phorate) 40 days after application. With allowances for variations, it seems that a period of 50 to 60 days should elapse between time of application and the time when animals are allowed to feed on the foliage after harvest. An interval as large as this appears compatible with a great majority of treatment needs in the Imperial Valley.

Discussion.—On the basis of these experiments, granulated phorate and Di-Syston appear to be promising materials for alleviation of the major pest problems affecting sugar beets in the Imperial Valley. It is difficult to determine with certainty the method whereby granulated systemics kill, following the topical method of application. On the basis of the relatively small amounts of toxicant found in the leaves (see table 6), it appears that toxicity may be largely a combination of fumigant and systemic activity. Toxicity resulting from pest contact with the granules appears to be minimal. As reported by Schopp & Landis (1959), Thiodan must have some toxic effect on the green peach aphid through fumigation (see table 4). There is no other readily apparent explanation for the aphid mortalities obtained with Thiodan granules.

Several areas in the western United States have reported, or suspect, resistance to organophosphorous insecticides in the case of the green peach aphid. Similar

Table 6.—Residues of granulated phorate and Di-Syston on sugar beet roots and tops sampled at intervals following topical application.

Treatment and Ounces per Acre	Days After Treatment Sampled	Sulfoxide Residue (P.P.M.)	
		Roots	Tops
	Experiment No. 3[a]		
Phorate, 13.5	8	N.D.[b]	0.77
	15	N.D.	0.30
	29	N.D.	0.07
	Experiment No. 4[a]		
Phorate, 16	7	N.D.[c]	—
Di-Syston, 16	7	N.D.	—
Phorate, 16[d]	68	N.D.	N.D.
Di-Syston, 16[d]	68	N.D.	N.D.

[a] For description of application, see preceding text under experiment numbers.

[b] Non-detectable. Minimum quantity detectable is 0.05 p.p.m. *O,O*-diethyl *S*-2-(ethylsulfinyl)methyl phosphorothiolate (phorate oxygen analogue sulfoxide).

[c] Non-detectable. Minimum quantity detectable is 0.66 p.p.m. *O,O*-diethyl *S*-2-(ethylsulfinyl)ethyl phosphorothiolate (demeton-thiol isomer sulfoxide).

[d] Special experiment applied on March 19, 1959, for study of residue only. Applications made to small plots, a single row wide and 50 feet long. Each treatment replicated four times. Applications were made with hand equipment in the manner described in Experiment Nos. 1 and 2.

resistance is suspected in the Imperial Valley. Certainly the results with demeton and parathion (see table 4) were poor. The coverage obtained on sugar beet plants as large as these is mediocre at best, however, and this may partially explain the poor results. Nevertheless, commercial applicators are experiencing increasing difficulty in attaining control and, consequently, are speculating on the possibility of resistance. Although phorate and Di-Syston are organophosphorous compounds, results to date have been excellent. If resistance in this area is or becomes a reality, the future use of these compounds for green peach aphid control in this area may be jeopardized.

In applying granulated materials in the manner utilized in these experiments, weather conditions should not be windy, and the plants should be dry; otherwise, too many granules adhere to the leaf surface.

Neither phorate nor Di-Syston granules had any effect upon larvae of the beet armyworm (*Spodoptera exigua* (Hbn.)).

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Effects of Timing and Method of Application on Efficiency of Co-Ral Sprays for Cattle Grub Control[1]

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ABSTRACT

When cattle were sprayed with 0.5% Co-Ral® (*O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate) at monthly intervals from May through September, there was no relationship between control and time of treatment. In other experiments with grown animals control ranged from 87.5% for ¾ gallon of 0.4% to 90% for 1 gallon of 0.5%, applied at a pressure of 300 pounds per square inch, but control obtained by treating calves lightly (approximately 1 quart) was only 31%. In further tests with 0.5% no differences were noted between ½ to 1½ gallons applied at 250 pounds and 1 gallon at 250 or 500 pounds per square inch. Except for the calves, cattle in all tests were in summer coat and appeared to be wet to the skin by the minimum volume and pressure.

During the last several years tests have been conducted in many sections of the country to determine the effectiveness of Co-Ral® (*O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate) for the control of cattle grubs (*Hypoderma lineatum* (De Vill.) and *bovis* (L.)). Results have been reported for tests in Texas (Brundrett *et al.* 1957, Drummond 1959), Louisiana (Burns & Goodwin 1958), Wyoming (DeFoliart *et al.* 1958), Mississippi (Neel 1958), Virginia (Turner & Gaines 1958), and in Texas with cattle from South Dakota (Graham 1958). All the reports indicated considerable variation in effectiveness, but most of them showed good to excellent control of grubs with dermal applications of ½ to 2 gallons of 0.5 or 0.75% Co-Ral. Results with lower concentrations were also variable but generally unsatisfactory.

The variability of results in Texas as well as in other states suggested that studies be conducted to determine the factors responsible. A series of experiments was conducted during the summer of 1958 to evaluate the effectiveness of Co-Ral at several times of treatment, concentrations, rates of application, and spray pressures.

TIME OF APPLICATION.—At the Texas Agricultural Experiment Station at Spur, 12 yearling Hereford steers were treated each month from May through September with 12 gallons of 0.5% Co-Ral with a power sprayer operating at a pressure of 250 pounds per square inch. Animals were examined for grubs at monthly intervals from December through February. As grubs encysted, their locations were marked on an outline map of the back of each animal, and cumulative counts were obtained.

The results, presented in table 1, indicate that all the treatments were highly effective. Although there were differences in control, they do not fit into a trend correlated with time of application.

VOLUME AND CONCENTRATION OF SPRAY.—Eleven herds of cattle near north Texas cities of Anson, Roby, and Spur were used in these tests. All herds were Hereford except one, which was Angus. They were maintained on pastures in accordance with local range practices. At each ranch one group of animals was treated and another group not treated. The two groups were placed in different pastures or identified so that they could be separated when examined for grubs. Cattle were sprayed with 0.4 or 0.5% Co-Ral at a volume of ¾ or 1 gallon per animal, with a power sprayer operating at a pressure of 300 pounds per square inch. At two ranches groups of calves 4 to 5 months old were sprayed lightly (approximately 1 quart) in accordance with current recommendations (Entomology Research Division 1959).

The animals were examined twice during the season of encystment of grubs. The examinations were more than 2 months apart. Since cattle were not individually marked, cumulative totals of grubs per animal were not obtained. Control was based on differences between average numbers of grubs in treated and untreated cattle (table 2). The results of four examinations are not included in further discussions or in the table because of small num-

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[2] P. T. Marion, superintendent of the Texas (Spur) Agricultural Experiment Station, C. Max Sturdevant, associate county agent for Fisher County, and Kirby Clayton, county agent for Jones County, assisted in these experiments.

Table 1.—Grub control in cattle sprayed monthly from April to September with 0.5% Co-Ral.

Date Treated	No. of Cattle		Average No. of Grubs per Animal	Control (%)
	In Test	Infested		
April 29	12	1	0.1	99
June 2	11[a]	5	.9	97
July 1	12	7	3.5	88
July 31	11[a]	5	1.0	97
September 3	12	9	5.1	83
Untreated	21	21	29.9	—

[a] One animal treated both times; grub data not included.

bers of grubs in controls or because too few cattle were examined.

As shown in table 2, the average control was 89%. There were no differences between ¾-gallon and 1-gallon treatments or between the 0.4 and 0.5% concentrations. However, the control afforded by lightly treating the calves was only 31%. Besides receiving a smaller dose of insecticide, the calves had hair coats that were considerably thicker and longer than those of the grown animals.

Spraying Pressure.—One herd of Hereford yearlings near Mountain Home was treated with 1 gallon of 0.5% per animal on July 23. Heifers were sprayed at a pressure of 250 pounds and steers at 500 pounds per square inch. The animals were examined for grubs twice during the season. Only a few animals were infested in October. As shown in table 3, both treatments afforded excellent control, and no differences in effectiveness were noted.

Table 2.—Grub control in cattle sprayed with different concentrations and volumes of Co-Ral.

Gallons per Animal	Date Treated	December Examination			February Examination		
		No. of Animals	Grubs per Animal	Control (%)	No. of Animals	Grubs per Animal	Control (%)
		0.4% Concentration					
0.75	July 1	45	1.8	85	45	1.1	80
0.25		20	8.2	34	0	—	—
	Untreated	8	12.5	—	10	10.9	—
1	July 2	38	1.2	84	34	0.9	—
	Untreated	18	7.2	—	6	10.2	91
		0.5% Concentration					
0.75	July 1	57	0.9	92	0	—	—
	Untreated	27	10.5	—	0	—	—
	July 29	—	—	—	41	0.4	95
	Untreated	—	—	—	7	7.7	—
	July 30	—	—	—	69	0.3	74
	Untreated	—	—	—	18	3.4	—
1	June 30	100	1.7	88	100	0.6	84
	Untreated	28	14.1	—	33	4.1	—
	July 29	44	0.2	95	46	0.3	92
	Untreated	19	4.0	—	19	3.7	—
1	July 29	32	1.0	88	—	—	—
0.25		11	5.9	28	—	—	—
	Untreated	15	8.3	—	—	—	—
1	July 30	137	0.7	93	98	0.4	91
	Untreated	39	10.9	—	23	5.3	—
	August 1	26	0.4	96	25	0.8	87
	Untreated	16	9.2	—	15	6.7	—
	August 1	—	—	—	10	0.7	84
	Untreated	—	—	—	6	4.3	—

Table 3.—Grub control in cattle sprayed with 0.5% Co-Ral at various volumes and pressures.

Gallons per Animal	Pressure (p.s.i.)	No. of Cattle		Average No. of Grubs per Animal	Control (%)
		In Test	Infested		
		Herd 1 (December examination)			
1	250	39	9	0.5	95
	500	54	10	.3	97
Untreated		22	22	11.2	—
		Herd 2 (Cumulative count)			
0.5	250	13	4	0.4	95
1		13	3	.4	95
1.5		8	3	.5	94
Untreated		12	11	7.9	—

Amount of Spray.—One herd of Hereford cattle near Kerrville was treated with 0.5% on June 25. Three groups were sprayed with different amounts per animal at a pressure of 250 pounds per square inch. Those treated with ½ gallon were fairly wet on top and sides, those with 1 gallon were well covered, and considerable insecticide ran off those treated with 1½ gallons. Cattle were examined for grubs at monthly intervals from September through January, and cumulative counts were obtained for each animal. The results, summarized in table 3, indicate that all treatments were highly effective in preventing encystment of grubs.

Discussion.—Results of experiments in timing of Co-Ral spray treatments agree with those obtained in Virginia by Turner & Gaines (1958) who found no differences in oral treatments of DOWCO 109 (*O*-(4-*tert*-butyl-2-chlorophenyl) *O*-methyl methylphosphoramidothioate) administered over a 5-month period, and in Texas by Wade & Colby (1958) who reported no differences with ronnel (Trolene) treatments from May through November. Apparently first-instar cattle grubs can be controlled by these three systemics at any time after the end of the heel fly season and before encystment of grubs. The seasonal occurrence of this period varies with the life cycle of cattle grubs in each area.

In north Texas control obtained with the least amount of Co-Ral (¾ gallon of 0.4%) was only slightly less than that obtained with the greatest amount (1 gallon of 0.5%). This finding indicates that sufficient toxicant to control grubs was absorbed from a thorough wetting with the smallest amount. However, only one herd was treated with this amount and treatments of large numbers of cattle may show more striking differences. Around Kerrville ½ gallon of 0.5% was 95% effective.

In general, the results of these experiments with concentration of Co-Ral, rate of application, and pressure agree with those of Brundrett *et al.* (1957), Burns & Goodwin (1958), Neel (1958), Turner & Gaines (1958), and Drummond (1959). The results were better than those obtained by Howell *et al.* (1958) and DeFoliart *et al.* (1958). The cattle treated in Louisiana, Mississippi, and Texas, including those in these experiments, were in summer hair coat, which is short and sparse. The cattle treated by DeFoliart and co-workers had the long heavy hair common to northern cattle in the fall and

winter. Cattle with similar coats were treated by Graham who used high pressures and volumes to penetrate the hair. Howell and co-workers considered inadequate pressure (225 pounds per square inch) one of the reasons that grub control was less for yearlings with long hair than for older animals with shorter hair treated in October-November.

In southern areas cattle are usually treated with systemics in the summer and early fall. With sprays the hair coat can be thoroughly wetted with ½ to 1 gallon and easily penetrated with a pressure of 250 pounds per square inch.

These studies point up the need for additional research to determine quantities of insecticide, amount of spray, and pressures needed for systemic grub control with Co-Ral sprays in cattle with different lengths of hair. It is apparent that, to insure absorption of enough toxicant, the method of application must vary according to climatic conditions.

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Mass-Production Methods for the Colonization of *Culicoides variipennis sonorensis*[1]

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ABSTRACT

A routine procedure for propagation of 1,000 adults per day is described. Viable-egg production has exceeded the number required to insure colony preservation. Colonization equipment and the procedures for handling adults are adequate for considerable colony expansion.

The methods used for the colonization of *Culicoides variipennis sonorensis* Wirth and Jones are presented in the hope that the interest of other workers will be stimulated so that research on the biology of this and other species of *Culicoides* will be expanded. The colonization of other species will not only increase our knowledge of *Culicoides* biology, but will assist in determining the role of these flies as vectors of animal diseases.

The colony (Sonora strain, from material collected on December 16, 1957) at the Kerrville, Texas, laboratory has averaged 1,000 adult flies per day for the past year. As only half the viable eggs produced are needed for maintenance at this strength, it is expected that the colony can be indefinitely continued at a level of high numbers. Also, with the production of large numbers of flies on a regular schedule, it has recently become possible to adopt a routine procedure for colony propagation.

An account of the methods first used for the colonization of this species, primarily the Sonora strain (Texas, Edwards Co., Texas A & M Substation No. 14, human sewage site at dwelling), has been published (Jones 1957). This colony died out after 12 consecutive generations in the laboratory, and large numbers of flies were never produced.

The life cycle in the laboratory is about 30 days—egg 2, larva 21, pupa 3, period prior to blood ingestion 1, and preoviposition period 3 days. The adults are held in a room maintained at 75° to 80° F. and a relative humidity of 40% to 50%. The larvae are kept at room conditions, where low temperatures are prevented by a thermostatically controlled stove, and even temperatures throughout the room are obtained with a blower system. All water used is hard well water without additives.

Weekday work requires practically the full time of a trained employee. Over the weekend a nonskilled employee can perform the essential tasks in a few minutes.

ADULTS.—*Equipment* (*Fig. 1*).—A holding cage is constructed of a 6-quart polyethylene container, a polyethylene funnel, and a 1¾-inch section of 3-inch diameter Plexiglas tubing. The three sections are joined and the inside joints sealed with electrician's rubber tape. One inch from the cage top there is a narrow slot for the insertion of a sheet-metal lid. A strip of white felt is cemented to the wall opposite the slot. The wall above the lid has a hole, covered by a thick rubber patch with a central slit, for anesthetization. There are four additional holes in the cage wall—two for aeration which are covered with 40-mesh wire screen, and two that are used in providing

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[2] G. H. Treiber constructed, and made many valuable suggestions concerning, the equipment used. Mrs. N. S. Allan was responsible for the maintenance of the colony. The photographs were taken by J. W. Warren.

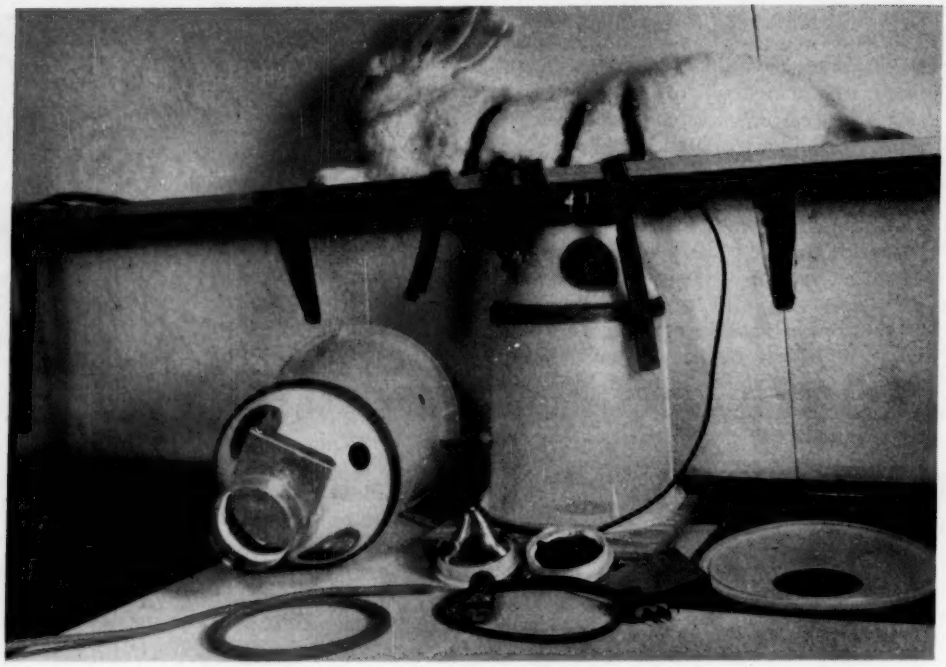

FIG. 1.—Holding and blood-feeding equipment.

sugar nourishment. The removable cage bottom is provided with three holes—a large one for the oviposition dish (or the pupal dish), which is a lid for a 250-ml. polyethylene beaker, and two small ones for the insertion of the tops of emergence cones. These cones are made of 40-mesh wire screen and are open at both ends.

A rabbit stock restrains four rabbits so that their closely clipped bellies sag through apertures 3¼ inches in diameter. The rapid placement of a rabbit is made possible by three leather straps that are adjustable in two directions through 1½-inch slots. A short metal tube with a light bulb inside is fastened with a rubber band under the stock at each aperture to direct light to the belly. Light intensity is regulated with a transformer.

Procedure.—Emergence cones, which fit into pupal dishes, are generally used for the introduction of pupae to holding cages. However, at the time a cage is set up, the pupal dish may be inserted in the large hole in the cage bottom. A population of 5,000 to 10,000 adults is maintained per cage. When the cage floor becomes heavily littered with dead flies, the live flies are anesthetized with carbon dioxide and transferred to a clean cage.

A 10% honey solution is afforded the flies through a cotton wick in a 2-dram lip vial. Raisins are compressed in a piece of cloth, and the twisted cloth is fastened through a hole in a cork. The honey is changed daily, and the raisins weekly.

Blood feeding of the females on a restrained rabbit is initiated 2 to 3 days after the first placement of pupae in a cage, and is repeated four times weekly. The cage is raised with large shims so that the upper rim fits snugly against the rabbit's belly, and the lid removed. The flies are fed with the room dark so that they are attracted to the belly by the dim light from the light device. After the flies have fed for 1 to 3 hours, they are jarred from the belly area and the lid is swiftly inserted. The few flies that are trapped are anaesthetized with carbon dioxide, recovered with an aspirator, and replaced in the cage.

A half-hour twilight period is furnished daily except weekends. Mating occurs frequently with flies 1 to 3 days old, and at this time the males commonly mate with females that are engorging with blood. Most mating occurs on a vertical strip of soft white paper that is spot-bonded to the cage wall, since most of the flies congregate in this area.

EGGS.—The oviposition dish is prepared by spreading mud evenly over all but the narrow margin of a paper disc that covers a thick layer of wet cotton in the dish. The paper disc facilitates the removal of the eggs. Each disc is labeled with the cage number and the date of the next day.

Oviposition dishes are changed every morning. Since eggs are cemented to the mud surface by the female, dead flies are washed off with a gentle stream of water to lessen the formation of mold during storage. By rapid microscopic examination the number of eggs on each disc is estimated, based on actual counts of eggs previously made. Only dark-brown eggs are recorded as viable. White to moderately brown eggs and those that are varicolored are considered sterile. Present viable-egg production is about 8,000 per day.

As soon as the eggs are collected, the discs with 1,000 or more viable eggs are stored on wet cotton in a tray in a refrigerator at 50° F. Discs with few eggs are generally used immediately. By a rotation system an ample supply can be stored and no batch remains under refrigeration too long.

LARVAE AND PUPAE.—*Equipment* (*Figs. 2 and 3*).—The larval pans are made of galvanized sheet metal and measure 30×25×2½ inches. The right rear corner of each

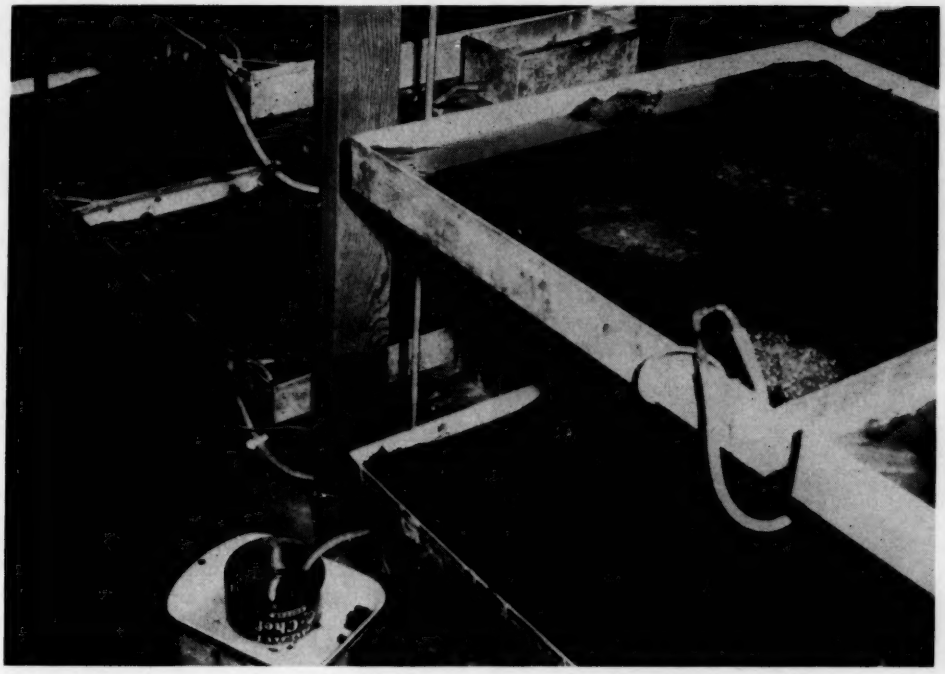

FIG. 2.—Larval rearing rack, close-up of larval pan.

pan is notched $\frac{3}{4}$ inch deep. The inside walls are painted white to facilitate observance of pupae. A pull-cord lamp that can be hooked above any pan provides light. A rack consisting of four shelves 18 inches apart holds 32 pans. At the rear of the rack two rows of four sun lamps each are operated during working hours.

The air and water systems are located at the rack front. Air is supplied by a rotary-vane compressor that operates continuously. The pressure is maintained at 12 pounds per square inch by a bleeder valve attached to the first of two 50-gallon buffer tanks that eliminate oil in the system. Pipes deliver air to both ends of the rack. Air is supplied by a $\frac{1}{8}$-inch rubber tube to each series of four pans. A three-way aquarium air valve regulates the flow at each pan. The tubing from each valve terminates in a pan with a piece of small-apertured glass tubing that is taped to a strip of metal bent at one end to form a clip over the pan edge.

The water system consists mainly of four horizontal lines, one beneath each shelf, which are connected by three vertical lines. Heavy-duty snaps are used to close the $\frac{1}{4}$-inch rubber tubing where water flow is not desired. Accurate leveling of the rack is necessary, as the water level is maintained by a central float for each shelf. A 5-gallon reservoir above the rack supplies the central float system and furnishes water to the vertical lines when desired. Since free flow of water is essential in the horizontal lines, the portions that lead to each pan and project above the water level are constructed of glass tubing so that air bubbles can be detected. A slightly slanted drain pipe is at the base of the rack. At each vertical drainage position this pipe is provided with a removable shallow funnel in which a 100-mesh sieve collects larvae from the drainage water.

At the rear of the rack is a slightly sloped trough that extends the full length of the rack and can be moved from shelf to shelf. A 100-mesh conical sieve is suspended under an aperture in the lower end of the trough. Rubber tubing from a funnel under this sieve discharges drainage water to the drain pipe below. A 100-mesh sieve in a shallow funnel attached to the drain pipe recovers larvae that are forced through the conical sieve by the water flow.

An upright 50-gallon barrel with an open top is used to recover larvae from discarded media. An overflow pipe at the rim and a drain at midheight open over a 100-mesh sieve placed in a funnel leading into a drain. Because of the great depth of water over discarded media, larvae swim to the surface and are flushed by a constant slight flow of water into the sieve below.

The device for estimating the number of pupae consists of a small funnel, a 50-ml. graduated cylinder, a U-shaped section of glass tubing containing water, a horizontal stopcock, and a rubber tube that is directed downward to a small sieve. All parts have large, nearly equal bores and are connected with rubber tubing.

Procedure.—The larval medium for one pan consists of 1 liter of cow manure, 2 liters of loose, black soil, one package of dried yeast, and about 2 liters of water. The manure is collected fresh from cattle maintained on pasture, as that of grain-fed cattle ferments readily. An ample supply is kept frozen in case fresh manure is not available; however, the larvae do not survive as well when frozen manure is utilized. The medium is stirred in a large mixer with water until it is loose but can be formed into mounds in the larval pan.

Two liters of clean sand is spread evenly in the pan, and the medium is added and formed into several gently sloped mounds. The right front corner of the pan is left clear for the entry of the air and water systems. Water is added to bring its depth to 1 inch from the pan bottom

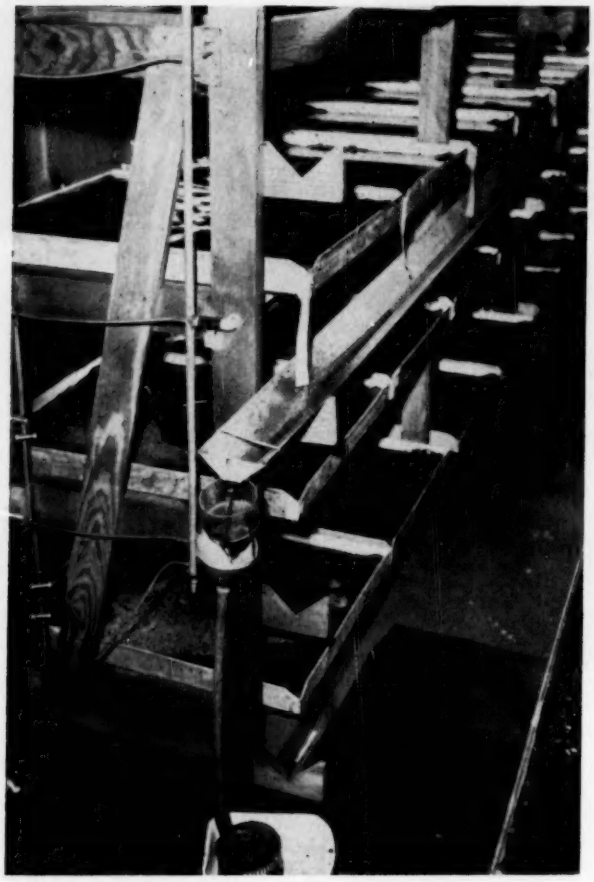

FIG. 3.—Larval rearing rack, pupal recovery system.

so that the mounds protrude about $\frac{1}{8}$ inch. The air tube, lying slightly beneath the surface, circulates the water with a flow of bubbles to prevent stagnation.

The egg discs are placed so that the eggs lie shallowly under water. About 4,000 viable eggs are placed on each pan. One pan is set up daily, and an additional one about every 3 days; pans for weekend use are set up in advance. The pans are left undisturbed until pupae form, except for daily checks for maintenance of the water level and circulation.

About 15 days after eggs are placed in a pan, room-temperature water from a 50-gallon tank is added to cover the mud areas. The pupae disengage themselves from the mud and float to the water surface. After about 2 hours the pan is flooded, so that water passes through the notch into the trough. A strip of masking tape attached under this notch directs the stream into the trough. Floating pupae are blown across the water through the notch by an air stream from a small-nozzled tube. After water addition has ceased and the pan is cleared of pupae, the pan is opened to the drain system, so that it can drain while others are being flooded.

To preserve most of the microorganisms present, for the first two or three floodings the fluid medium is drained off and returned to the pan after flooding is completed. As flooding leaches a large amount of the nutritive material from the medium, a small amount of manure is added to each flooded pan later. The pan is allowed to drain completely if it is ready for discard or if the original fluid medium is to be restored. Pans that have been flooded for 1 to 2 weeks are discarded at a rate designed to keep the number being flooded on 1 day from 6 to 10 pans.

As the pupal stage lasts $2\frac{1}{2}$ to 3 days, pans are flooded every other day during the working week. There is a slight loss of pupae over weekends owing to adult emergence. No attempt is made to recover the few pupae that might be present on the first day or two of pupal formation.

The material collected from the trough is flushed with water to remove foam and placed in a 35-mesh sieve slanted through the surface of the water in a shallow pan. The larvae pass through the screen into the pan. This procedure is repeated several times until they are separated from the pupae. These larvae, together with those collected as a result of other procedures for recovering pupae, are placed in a 60-mesh sieve and the small larvae are separated from the large in the same manner. The small larvae are placed in larval pans that have been held about 10 days; the large larvae retained in the sieve are placed in pans held about 15 days or in a pan freshly set up for this purpose.

The pupae are flushed into a pan of water, where most of the debris settles out. The large, floating debris is quickly removed, and the pupae are placed in the device for estimating the number of pupae. The level of the water in the vertical column is adjusted with the stopcock so that the floating pupae pack into a band in the graduated cylinder. A reduction of one-tenth in a reading is required to allow for debris present. (There are about 1,000 clean pupae per milliliter.) The stopcock is then released, and the pupae are flushed into the sieve and washed onto cotton in a pupal dish.

DISCUSSION.—The colonization equipment described can easily be modified to rear much smaller numbers of flies than 1,000 per day. It is also sufficient to sustain a colony of much greater size. Procedures for handling the adult flies are adequate for expansion, but techniques for larval rearing are not. In the field larvae of *variipennis* will breed in much greater concentrations than occur in colony pans.

Since the egg supply has recently exceeded the number required to insure preservation of the colony, experimentation towards an improved larval medium can be accelerated. Previous tests with improved media have yielded greater numbers of secondary breeders but not of *variipennis*. An enriched medium is of little value if enough eggs to increase the culicoid population are not available. As indicated by natural habitats, a dense population of *variipennis* in an enriched medium might eliminate secondary breeders by crowding. The principal secondary breeders have been species of the dipterous families Tendipedidae and Psychodidae. Recently dense populations of bloodworms (Tendipedidae) on larval pans temporarily reduced the colony to about 200 adults per day. It was difficult to eliminate these secondary breeders, because they have a much shorter life cycle than *variipennis* under existing colonization procedures. Also, the rearing room was not insect-proof, and consequently was exposed to reinfestation.

The colony has become adapted to laboratory conditions after 2 years of continuous propagation that covers 24 to 48 generations. An indication of this adaptability is that during the last year the percentage of females taking a blood meal has increased so that a simplified method for providing blood meals could be adopted. This method takes advantage of the fact that the flies readily fly or climb to the blood-feeding surface at the top of the cage. Previously a low percentage of females engorged regardless of the method used.

As large numbers of larvae and adults were needed during the past year for larvicide tests and for disease transmission trials, the colony was expanded even though some of the rearing procedures were known to be inadequate. Biological experimentation for better methods of propagation has necessarily been curtailed. The colony has periods of low productivity, often for no apparent cause, both in respect to larval survival and egg deposition. Some of the major colonization procedures that are of doubtful efficacy should be mentioned. The holding cages for adults that are described are not fully proved. A smaller cage made of a 1-gallon ice cream carton with a cone of fine-mesh wire screen has frequently been used with greater success during periods when production was low. Tests with sun lamps have been inconclusive and do not indicate a definite need as anticipated. Several paired tests, in which the estimated number of viable eggs deposited was compared with the number of pupae used, indicated that a twilight period was of only slight value in increasing egg production. It is not clear whether the oxygenation of the water in the larval pans is advantageous.

REFERENCE CITED

Jones, Robert Henry. 1957. The laboratory colonization of *Culicoides variipennis* (Coq.). Jour. Econ. Ent. **50**(1): 107–8.

Three Plans for Using Acaricides to Control Mites on Apple[1]

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ABSTRACT

Certain acaricides were tested in 1958 and 1959 as control agents for the European red mite, *Panonychus ulmi* (Koch), and the two-spotted spider mite, *Tetranychus telarius* (L.), according to three plans: (1) single acaricides alone at standard concentration in consecutive acaricidal applications; (2) two acaricides separately at standard concentration in alternate applications, and; (3) two acaricides mixed, with each at one-half standard concentration, in consecutive applications. Test data indicate that plan 3 is better than plan 2. The data also indicate that mixtures of two acaricides, with each at one-half standard concentration, give better control than the weaker of the two applied alone at standard concentration.

In the spring of 1958, an experiment was designed with the objective of learning if acaricides might select resistant races of mites at different rates when applied according to the following three plans:

1. One acaricide used alone at so-called standard concentration in consecutive acaricidal applications.
2. Two acaricides used separately at standard concentration in alternate acaricidal applications.
3. A mixture of two acaricides employing each at one-half standard concentration, with the mixture applied in consecutive acaricidal applications.

METHODS OF MATERIALS.—The experiment was laid out in a block of 10-year-old apple trees in the Station orchard near Arendtsville. Five rows of trees were chosen, one row of each of the following varieties: York Imperial, Rome Beauty, Golden Delicious, Stayman and Delicious. Each row was a replicate. Single tree plots were employed in an arrangement that left a buffer tree between each pair of plots.

The acaricides employed in 1958 and 1959 are listed in tables 1 and 2. The original plan was to use the same acaricides, Aramite® (2-(*p-tert*-butylphenoxy)-1-methylethyl 2-chloroethyl sulfite), Kelthane® (4,4′-dichloro-*alpha*-(trichloromethyl)-benzhydrol), and Tedion® (2,4,4′,5-tetrachlorodiphenyl sulfone) throughout the experiment. However, after 1958 Aramite was replaced by dimethoate because the residue tolerance for Aramite on food crops was cancelled. In both 1958 and 1959, the same trees were used for the treatments as numbered in the tables. Hence, only those 1959 treatments in which dimethoate was substituted for Aramite differ from 1958 treatments of the same number.

The acaricide treatments were applied with a spray gun operated from a high pressure sprayer. All other sprays were applied as mist concentrates. The same general sprays were used on all the varieties except that in one or two prebloom sprays glyodin was applied to the Delicious trees instead of captan to avoid injury to the foliage. The pesticides employed in the general purpose sprays during the summer period were for the most part: captan, zineb, Sevin® (1-naphthyl methylcarbamate), TDE, DDT and lead arsenate.

No prebloom sprays of acaricides were applied to the trees in this experiment. In fact, acaricides were not employed until the mite population showed signs of making a typical summer upsurge in numbers.

In both seasons, the mite population was a mixture of the European red mite, *Panonychus ulmi* (Koch), and the two-spotted spider mite, *Tetranychus telarius* (L.). The European red mite predominated until mid-July in both seasons. Then, the two-spotted spider mite predominated throughout the balance of the period counts were made,

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Table 1.—Control of a mixed population of European red mites and two-spotted spider mites based on the average number of mites per leaf accumulated in five postspray counts[a] from five replicates of each treatment. 1958.

Treatment No.	Spray Dates and Acaricides[b] (Lb./100 Gal.) July 8	July 23	Prespray Mites/Leaf July 3	Postspray Average Accumulated Mites/Leaf	Per Cent Control[c]
1	Aramite 2	Aramite 2	9	19.8	87.3
2	Tedion 0.5	Tedion 0.5	7	28.6	81.7
3	Aramite 1 Tedion 0.25	Aramite 1 Tedion 0.25	6	16.4	89.5
4	Aramite 2	Tedion 0.5	7	19.4	87.6
5	Kelthane 2	Kelthane 2	6	8.6	94.5
6	Kelthane 1 Aramite 1	Kelthane 1 Aramite 1	12	8.4	94.6
7	Kelthane 2	Aramite 2	3	22.2	85.8
8	Kelthane 1 Tedion 0.25	Kelthane 1 Tedion 0.25	5	11.0	93.0
9	Kelthane 2	Tedion 0.5	3	18.6	88.1
10	Check—No acaricides		2	156.0[d]	
L.S.D. at 5% level				2.70	
1% level				3.62	

[a] Postspray mite counts on July 21 and 28; August 5, 11 and 19.
[b] Weights of the acaricides per 100 gallons are for wettable powders containing the following amounts of active ingredients: Aramite, 15%; Tedion, 25%; Kelthane, 18.5%
[c] Per cent control was calculated according to Abbott's formula, using the figures for the average number of accumulated mites only in comparison with the check.
[d] Check figures not used in analysis.

Table 2.—Control of a mixed population of the European red mite and the two-spotted mite based on the average number of mites per leaf accumulated in seven postspray counts[a] from five replicates of each treatment. 1959.

Treatment No.	Spray Dates and Acaricides[b] (Lb./100 Gal.) June 19	June 30	July 24	Prespray Mites/Leaf June 16	Postspray Average Accumulated Mites/Leaf	Per Cent Control[c]
1	Dimethoate 0.5	Dimethoate 0.5	Dimethoate 0.5	24	22.6	92.6
2	Tedion 0.5	Tedion 0.5	Tedion 0.5	9	53.8	82.3
3	Dimethoate 0.25 Tedion 0.25	Dimethoate 0.25 Tedion 0.25	Dimethoate 0.25 Tedion 0.25	25	26.0	91.4
4	Dimethoate 0.25	Tedion 0.5	Dimethoate 0.5	29	31.8	89.5
5	Kelthane 2	Kelthane 2	Kelthane 2	26	14.2	95.3
6	Kelthane 1 Dimethoate 0.5	Kelthane 1 Dimethoate 0.25	Kelthane 1 Dimethoate 0.25	29	13.0	95.7
7	Kelthane 2	Dimethoate 0.5	Kelthane 2	33	17.4	94.3
8	Kelthane 1 Tedion 0.25	Kelthane 1 Tedion 0.25	Kelthane 1 Tedion 0.25	30	23.6	92.2
9	Kelthane 2	Tedion 0.5	Kelthane 2	17	29.0	90.4
10		Check—No acaricides		30	303.2[d]	
L.S.D. at 5% level					10.47	
at 1% level					14.04	

[a] Postspray mite counts on June 29; July 9, 13 and 27; August 3, 10 and 20.
[b] Weights of acaricides per 100 gallons are for wettable powders containing the following amounts of active ingredients: dimethoate, 50%; Tedion, 25%; Kelthane, 18.5%.
[c] Per cent control calculated according to Abbott's formula, using the figures for the average number of accumulated mites only in comparison with the check.
[d] Check figures not used in the analysis.

with the European red mite tending to increase in numbers at the time of the last count about August 20.

Motile forms of the mites only were counted directly on the leaves with the aid of a 7× lens. No attempt was made to count mites that could not be seen clearly with this lens. The mites were counted on 20 leaves per replicate each time. This gave a total of 100 leaves per treatment as the basis of each count. As shown in tables 1 and 2, this system yielded valid data.

DISCUSSION OF RESULTS.—The results from this experiment, as presented herein, are offered not to answer the original objective but to supply information that may be useful in connection with the resistance problem. Details of the results will be found in tables 1 and 2. In each table, the figures in the column headed "Postspray Average No. of Accumulated Mites/Leaf" were arrived at as follows: the numbers of mites per leaf counted in each replicate of each treatment throughout the summer were added together. Then, these totals for the replicates of each treatment were added together to make a grand total for the treatment. Finally, this figure was divided by 5, the number of replicates, to give the average number of accumulated mites per leaf for the treatment. The figures for the replicates were, of course, used in the analysis of variance.

CONCLUSIONS.—From the results of this experiment, it may be concluded that mixtures of two acaricides, each at one-half standard concentration, in consecutive applications tend to give better control of the European red mite and the two-spotted spider mite than alternate applications of the same acaricides at standard concentration. In 1958 and 1959, mixtures of two acaricides, each at one-half standard concentration, in consecutive applications were compared in six cases with the same pairs of acaricides applied alternately at standard concentration. The mixtures gave better results in all six cases, significantly at the 1% level in two cases and at the 5% level in one instance.

Also, it may be concluded that mixtures of two acaricides, each at one-half standard concentration, in consecutive applications tend to give better control of these mites than the weaker of the two acaricides does when used alone at standard concentration in consecutive applications. In six such comparisons in 1958 and 1959, the mixed acaricides gave better results in all six cases, significantly at the 1% level of probability in five cases.

COMMENTS.—All the acaricides and mixtures of acaricides employed in this experiment gave satisfactory control from the standpoint of protecting the trees from mite injury. However, the data indicate that standard concentration for both dimethoate wettable powder and Tedion wettable powder should be set at about 12 ounces per 100 gallons of spray in future tests to bring the standard concentration for them to a par with 2 pounds of Kelthane wettable powder in 100 gallons. None of the acaricides tested caused observable injury to the fruit or foliage of the five varieties of apples used in this experiment.

Host Range and Damage by the Grass Mealybug, *Heterococcus graminicola*[1]

SHERL M. DIETZ[2] and ROBERT F. HARWOOD[3]

ABSTRACT

The known host range of the grass mealybug (*Heterococcus graminicola* Morr.) has been increased from six grass species in six genera and two cereals (wheat and corn) to 67 species and one variety representing 23 genera. Mealybug infestations of commercial stands of Merion bluegrass and creeping red fescue were also observed. These infestations were especially severe following insecticide applications for control of webworms and cutworms. Brief descriptions and pictures of the insects and various symptoms of affected hosts (browning of leaf sheaths, leaf tip dieback, and whitehead conditions) are presented. Known aspects of the grass mealybug life history and suggested controls are also included.

Infestation of a grass mealybug in a seed field of Merion bluegrass was noted in Garfield county, Washington, in the summer of 1955. The infestation was characterized by browning of leaf sheaths and general unthriftiness of plants. Similar conditions were noted subsequently in seed fields of Merion bluegrass and creeping red fescue in Spokane county, particularly following insecticide applications to control webworms and cutworms. The mealybug involved was identified by Dr. Harold Morrison.[4] He and L. G. Davis of the Plant Pest Control Division of the Agricultural Research Service provided previous records of infestation on six species of grass, on wheat, and on the adventitious roots of corn. Records show that the known distribution of the insect is scattered, it being reported from states on the eastern and western seaboard, and Utah, Minnesota and Ohio.

During the summer of 1957 an increased leaf tip dieback was noted on many grasses in the Regional Plant Introduction and Soil Conservation Service nurseries, Pullman, Washington. Examination revealed the grass mealybug, *Heterococcus graminicola* Morr., under the sheaths of such affected leaves (figure 2). Since many species of grasses were present, a study of grass hosts and symptoms of injury was undertaken.

THE GRASS MEALYBUG.—*H. graminicola* Morr. (Figure 1.)—Adult females are 4 mm. or less in length. The body is noticeably segmented, elongate-ellipsoid, and flattened dorsoventrally. Adults and nymphs are yellowish to orange, but this color is often obscured by a white cottony substance surrounding the adults. The eggs which

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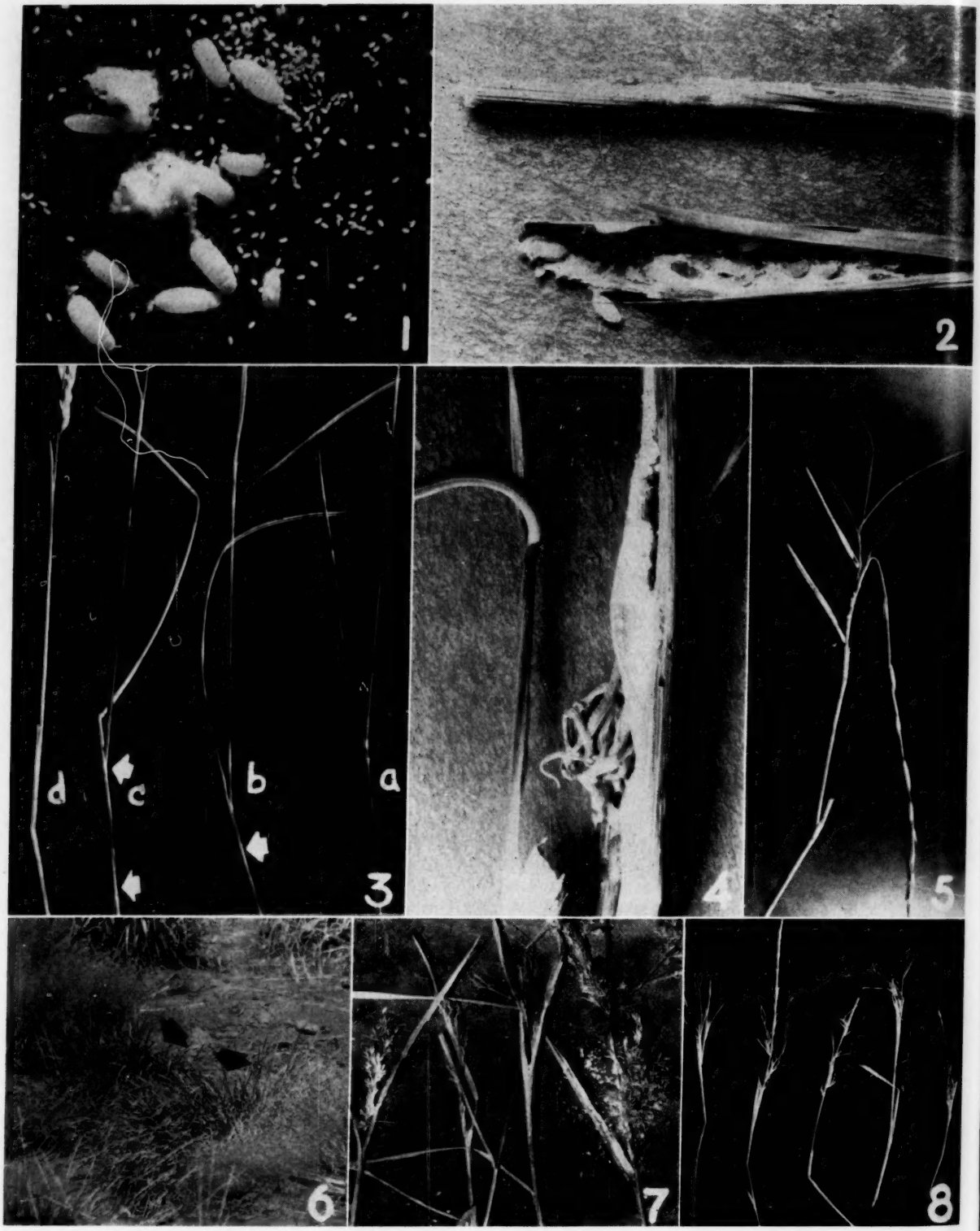

FIG. 1.—Adult female mealybugs (*Heterococcus graminicola*), large nymphs, eggs and cottony material, collected from *Hordeum bulbosum* (P.I. 206372).

FIG. 2.—The leaf sheath (lower) removed from the culm (upper) of *Festuca arundinacea* showing mealybug habitat.

FIG. 3.—(a) Healthy uninfested culm of *Festuca arundinacea* (P.I. 235036) on the right; (b, c and d) three infested culms on the left illustrating varying degrees of mealybug injury; (b) feeding just below the collar causing death of the sheath tissue (white arrow) and leaf tip; (c) feeding well below sheath collar causing increased sheath damage (white arrows) and leaf nearly dead; (d) sheath

are barely visible to the unaided eye, appear as minute yellowish dots and are often embedded in the cottony substance. See H. Morrison (1945) and G. F. Ferris (1953) for further description.

HOST RANGE.—Although previous reports indicated a rather limited number of Gramineae as hosts, a host survey at the Plant Introduction nursery revealed annuals and perennials of 23 grass genera, embracing 67 species and one variety. A more extensive study probably would add materially to the number of new hosts. Since mealybug-infested corn and bristlegrass were not observed, *Zea* and *Setaria* are not included in the list, even though previous reports show both as hosts. For convenience the genera, as well as the species within each genus, are listed alphabetically.

Agropyron
- *buonapartis* (Spreng.) Dur. & Schinz.
- *caespitosum* Koch
- *cristatum* (L.) Gaertn.
- *dasystachyum* (Hook.) Scribn.
- *elongatum* (Host) Beauv.
- *inerme* (Scribn. & Smith) Rydb.
- *intermedium* (Host) Beauv.
- *latiglume* (Scribn. & Smith) Rydb.
- *pseudorepens* Scribn. & Smith
- *repens* (L.) Beauv.
- *smithii* Rydb.
- *spicatum* (Pursh) Scribn. & Smith
- *subsecundum* (Link) Hitchc.
- *trachycaulum* (Link) Malte
- and unidentified spp.

Agrostis
- *castellana* Boiss & Reut.
- *tenuis* Sibth.
- and unidentified spp.

Alopecurus
- *aequalis* Sobol.
- and unidentified spp.

Arrhenatherum elatius (L.) Presl

Bromus
- *anomalus* Rupr.
- *carinatus* Hook. & Arn.
- *ciliatus* L.
- *erectus* Huds.
- *inermis* Leyss.
- *marginatus* Nees
- *tomentellus* Boiss
- *trinii* Desv.
- *vulgaris* (Hook.) Shear
- and unidentified spp.

Cynosurus cristatus L.

Deschampsia sp.

Dactylis
- *glomerata* L.
- *glomerata var. hispanica* (Roth) Koch

Danthonia sp.

Elymus
- *glaucus* Buckl.
- *innovatus* Beal

Festuca
- *ampla* Hackel
- *arundinacea* Schreb.
- *elatior* L.
- *ovina* L.
- *pratensis* Huds.
- *rubra* L.
- *scabrella* Torr.

Haynaldia hordeacea (Coss. & Dur.) Hackel

Helictotrichon pubescens (Huds.) Pilger

Hordeum
- *bulbosum* L.
- *californicum* Covas & Stebbins
- *pusillum* Nutt.
- *vulgare* L. (Barley.)

Koeleria sp.

Lolium perenne L.

Melica
- *imperfecta* Trin.
- and unindentified spp.

Phalaris arundinacea L.

Phleum
- *alpinum* L.
- *pratense* L.

Poa
- *alpina* L.
- *ampla* Merr.
- *annua* L.
- *artica* R. Br.
- *bulbosa* L.
- *canybi* (Scribn.) Piper
- *compressa* L.
- *glauca* Vahl
- *palustris* L.
- *pratensis* L.
- *stenantha* Trin.
- *trivialis* L.
- and unidentified spp.

Secale montanum Guss.

Sitanion hystrix (Nutt.) J. G. Smith

Triticum aestivum L. (wheat)

The 1957 Plant Introduction nursery contained 317 rows of grasses. In 1958, 308 rows, or 97% of the rows of grasses in this nursery, were infested. No mealybugs were observed on the following nine lines:

229914[5]	Agropyron caespitosum	Iran[6]
229910	Agropyron sp.	Iran
232139	Agropyron spicatum	Montana

[5] Plant Introduction number.
[6] Area from which the seed was originally collected.

nearly dead and dead leaf whipped off by wind. Removal of the sheath at stages illustrated in c and d usually reveals many mealybugs as seen in figure 2.

FIG. 4.—Aborted mealybug-infested inflorescence of *Hordeum* (right), close up of the "bends" area (left) of an *Agropyron* culm.

FIG. 5.—*Agropyron trachycaulum* (P.I. 236712) infested with mealybugs, illustrating a leafy-shoot characteristic along with the "bends."

FIG. 6.—Row of *Agrostis tenuis* (P.I. 235217). The center two plants (black arrows) are heavily infested with mealybugs and produced only aborted heads. Notice prolific heading of plants (left rear) with very few mealybugs.

FIG. 7.—Close-up of aborted heads taken from *Agrostis tenuis* plants shown in the center of figure 6. The heads were pulled out from their enveloping sheaths showing heavy mealybug infestation (white spotting). The head at the right was one of a few reaching this stage of development; most others never set seed.

FIG. 8.—*Agropyron trachycaulum* (P.I. 236712) showing proliferated leafy shoots associated with heavy mealybug infestations.

232120	Agropyron smithii	Montana
233819	Dactylis glomerata	Italy
234689	Dactylis glomerata	Denmark
229455	Festuca sp.	Iran
234757	Festuca rubra	Spain
234753	Festuca rubra	Spain

Some heavily infested lines were:

236678	Agropyron smithii	Canada
236708	Agropyron trachycaulum	Canada
236712	Agropyron trachycaulum	Canada
236720	Agropyron trachycaulum	Canada
236721	Agropyron trachycaulum	Canada
236722	Agropyron trachycaulum	Canada
236728	Agropyron trachycaulum	Canada
235217	Agrostis tenuis	Sweden
229427	Alopecuris sp.	Iran
236732	Alopecuris aequalis	Canada
232233	Bromus marginatus	Colorado
234720	Festuca arundinacea	France
235036	Festuca arundinacea	Denmark
234777	Festuca elatior	Germany
206372	Hordeum bulbosum	Cyprus
234697	Phalaris arundinacea	Denmark
234780	Phalaris arundinacea	Germany
234796	Phalaris arundinacea	Sweden
236898	Poa ampla	Canada

SYMPTOMATOLOGY.—Active nymphs crawl over all plant parts, generally enter the space between leaf sheath and culm at the collar, and progress varying distances down between the sheath and culm (fig. 2). Here they commence to feed on the outer portion of the culm and the inner portion of the leaf sheath. The first noticeable symptom is a slight brownish discoloration of the outer sheath tissue, generally at the collar area, but occasionally lower if the insects descend beyond the collar (fig. 3b, 3c, white arrows). They then multiply, but since the adult females are more or less sedentary, a great deal of feeding is concentrated in the area of the leaf sheath. This local feeding leads to further sheath tissue breakdown, which in turn is followed by a progressive die-back and shriveling of the leaf tip (fig. 3b, 3c). In heavy infestations the insects may be found under all the leaf sheaths of each culm as well as among basal leaves. The region of the inflorescence may be attacked while still in the "boot" stage, causing the inflorescence to be malformed and unproductive (figures 4, 6, 7). On occasion feeding kills the culm after emergence of the inflorescence, resulting in a dead inflorescence or "whitehead." This whitehead condition is especially prevalent on the bluegrasses but may also be found on species of other genera. In general, mealybug infestation of grass produces an unthrifty appearance. The proliferation of leaf-like structures arising in place of the florets (figures 5, 8) occurred in several lines of *Agropyron trachycaulum*. This proliferation was found only in the presence of abundant mealybugs. The possibility of these insects producing a growth-stimulating substance, or merely an injury stimulus, remains to be explored, as does the possibility of their being virus vectors.

Fischer (1941) reported a "bends" disease of grasses of unknown cause. The authors observed that this disease occurred along with mealybug infestation, but no evidence has been found linking the two (figures 4, 5).

Heavy infestations, particularly on bromes, often are associated with increased susceptibility of the host to such diseases as rust, leaf spots, and powdery mildew. However, several lines of *Festuca arundinacea* were heavily infested with mealybugs, but otherwise appeared disease-free.

LIFE HISTORY.—This mealybug of grasses (*H. graminicola*) is often overlooked because it is usually located under the leaf sheaths. Nymphs, which are active crawlers, may be observed on the upper foliage during March in the area of Pullman, Washington. These nymphs crawl from plant to plant and their spread in the field is amazingly rapid considering the fact that the adult females move very little. Spread is demonstrated by the fact that large second-year nurseries were 97% infested and first-year nurseries, along with annuals, such as wheat and barley, also became infested. The winged males, illustrated by Morrison (1945), were not observed. Active nymphs become sedentary females under the leaf sheaths, where they produce a white cottony substance and lay eggs (figure 2). The number of days from egg to egg and generations per year have not been determined. Either the total population decreases or most of the insects moved to lower, more protected areas as the plants mature during hot midsummer months. However, some live mealybugs can still be found beneath the upper leaf sheaths and in inflorescences after the first fall frost. In severe winters the only stages noted are very young nymphs which are generally located in protected locations at the base of the plant, even below the soil at the upper root level. Occasional eggs and adults are found in winter, but these apparently are not viable.

CONTROL.—Late-summer and fall burning reduces overwintering populations of these mealybugs. Thus far no observed infestation of mealybugs has caused sufficient damage to warrant the expense of insecticides. Experiments indicate that an application of malathion at 1 pound per acre early in the spring (March), when young crawlers are actively moving about on the plants, and before most of them have become established in protected areas, is effective. Phorate or Trithion® (*S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate) at ½ pound per acre is also effective under similar conditions.

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Further Studies on the Toxicity of Insecticides to Diazinon-Resistant *Musca domestica*[1]

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ABSTRACT

A strain of house fly, *Musca domestica* L., which had developed resistance to Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate) in the field and then was subjected to further selection on Diazinon in the laboratory, showed increased tolerance to the following materials: heptachlor (468-fold), heptachlor epoxide (809), barthrin (3), demeton (12), phorate (17), dimethoate (3), DDVP (4), dicapthon (18), Dibrom® (1,2-dibromo-2,2-dichloroethyl dimethyl phosphate) (4), Phosdrin® (1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate) (7), Am. Cyanamid 18706 (*O,O*-dimethyl S(N-ethyl carbamoyl methyl) phosphorodithioate (technical) (5), Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide) (58), Bayer 29493 (*O,O*-dimethyl *O*-(4-methylthio-*m*-tolyl) phosphorothioate) (8), G-30494 (*S*-2(2,5-dichlorophenylthio)methyl *O, O*-dimethyl phosphorodithioate) (12), Dimetilan® (3-methyl-5-pyrazolyl dimethylcarbamate) (6), Dipterex® (dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate) (7), Delnav® (2,3-*p*-dioxanedithiol *S,S*-bis (*O,O*-diethyl phosphorodithioate) (89), DMBC (2,4,-dimethyl benzyl chrysanthemumate) (3), and Thanite® (isobornyl thiocyanoacetate) (34-fold).

The first observations of cross-resistance in Diazinon® (*O,O,*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate)-selected strains of house fly, *Musca domestica* L., were made in Switzerland in 1953 by Kocher (1955, 1956). Since then, laboratory and field studies with Diazinon have demonstrated an extensive pattern of cross-resistance, not only to other organophosphorus insecticides but to a variety of structurally unrelated compounds as well. Many of these reports have been summarized by Brown (1958) and Forgash & Hansens (1959).

The strain of house fly utilized in the present study originally developed resistance in a New Jersey dairy barn (Hansens 1958). Failure of control with Diazinon had become evident after 5 years of successful fly management, involving a total of nine treatments with 1% material. Laboratory panel tests showed that the strain was resistant not only to Diazinon but to other insecticides. After further selection in the laboratory, topical application tests demonstrated complete insensitivity to DDT-type compounds, very high resistance to chlorinated cycloalkanes, high resistance to Diazinon (38-fold), moderate resistance to other phosphorothioates (16- to 23-fold), to carbamates (10- to more than 37-fold), and to Dilan® (1 part of 1,1-bis(*p*-chlorophenyl)-2-nitropropane plus 2 parts of 1,1-bis(*p*-chlorophenyl)-2-nitrobutane) (10-fold). The strain had also developed tolerance to malathion (5-fold), allethrin (5-fold), pyrethrins (4-fold), and Lethane 384 (2-(2-butoxyethoxy)ethyl thiocyanate) (2-fold) (Forgash & Hansens 1959).

At this point it is well to note that the resistance history of this strain in the field in all probability predates the use of Diazinon. In 1951, flies from this same barn were resistant to methoxychlor (Hansens 1958) and undoubtedly to other DDT analogues. Also, it is possible that some degree of resistance was present to cycloalkanes despite the fact that such materials never had been used in that particular barn, for Hansens (1953) has shown that the general area contained populations moderately resistant to dieldrin, heptachlor, and lindane.

Although the level of resistance to the chlorinated hydrocarbons at the time Diazinon treatments were initiated in the field is not known, there is substantial evidence that the present magnitude is a direct result of selection with Diazinon (Forgash & Hansens, unpublished results).

Serious concern over the threat that cross-resistance of the type exhibited by this strain of fly presents to house fly control, if not eventually to insect management in general, has prompted this exploration of the resistance spectrum in immediate quest of effective control materials, and eventual understanding of the basic principles involved. Such information will also be of considerable value to current studies on the fate of resistance both under continued Diazinon pressure and in the absence of further selection, and on the mechanism of resistance to Diazinon and other materials.

EXPERIMENTAL RESEARCH. Dosage-mortality data for various insecticides were obtained from topical application of several concentrations in 2 μl. of acetone (A.C.S. grade) solution, prepared within 24 hours of testing. The house fly strain was a Diazinon-resistant colony that had been collected in the field and selected further on Diazinon-treated panels in the laboratory. At the time of testing, the Diazinon-resistance level of the strain was fairly constant, although the strain was still under selection (1 hour on 10 mg. Diazinon per square inch of plywood).

Unsexed 4-day-old flies were used and each material was tested simultaneously on a laboratory susceptible-strain (Wilson line). For treatment, the flies were immobilized with CO_2 and placed in a petri dish, which contained a piece of dental wick saturated with skim milk. Each dish contained 10 flies and a total of 30 flies was used for each concentration of insecticide. The materials were evaluated at five or more concentrations and each test was repeated at least twice and on different days. Mortality readings were made 24 hours after treatment, all flies incapable of locomotion being considered as dead.

Log dosage-probit (ld–p) lines were fitted to the data by the method of least squares, and the corresponding LD50s, LD95s, and regression coefficients were calculated.

The insecticides tested were: Diazinon (94.5%), heptachlor (purified), heptachlor epoxide (purified), barthrin

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Table 1.—Toxicity of various insecticides to a Diazinon-selected (field and laboratory selections) resistant strain and a susceptible strain (Wilson) of house fly.

Insecticide[a]	Susceptible Strain		Resistant Strain			
	LD50 (μg./gm.)	LD95 (μg./gm.)	LD50 (μg./gm.)	Increase in LD50	LD95 (μg./gm.)	Increase in LD95
Diazinon	2.52	6.60	220	87x	700	106x
Delnav	18.6	68.4	1,650	89x	7,600	111x
Dimethoate	0.840	2.88	2.50	3x	8.90	3x
Am. Cyanamid 18706	4.14	11.2	19.8	5x	59.5	5x
Dipterex	14.4	51.6	104	7x	263	5x
DDVP	1.08	2.28	4.20	4x	9.35	4x
Thanite	525	1,720	18,100	34x	155,000[b]	90x
Dimethyl benzyl Chrysanthemumate	46.5	134	128	3x	357	3x
Dicapthon	1.68	6.06	30.0	18x	123	20x
Barthrin	20.9	67.8	63.0	3x	162	2x
Phorate	5.76	12.4	95.0	17x	361	29x
Demeton	25.7	48.8	300	12x	1,050	22x
Thiodan	4.80	12.5	277	58x	1,500	120x
Heptachlor	0.534	1.40	250	468x	228,000[b]	160,000x
Heptachlor epoxide	0.408	1.07	330	809x	465,000[b]	440,000x
Dibrom	1.26	3.36	4.90	4x	11.7	3x
Dimetilan	8.52	28.7	55.0	6x	370	13x
Phosdrin	1.44	3.48	10.0	7x	36.0	10x
Bayer 29493	4.80	7.56	33.0	8x	39.0	12x
G-30494	5.70	31.8	66.5	12x	288	9x

[a] Dosages are based on the sample used for dilution, regardless of its purity, except in the case of demeton where the dosage refers to 100% insecticide and not the 20% material.

[b] These values were obtained by extrapolation; the highest mortality obtained with Thanite was 83% and with heptachlor and heptachlor epoxide, 70%.

(technical), demeton (20.2%), phorate (99%), dimethoate (technical), DDVP (technical), dicapthon (technical 96%), Dibrom® (1,2-dibromo-2,2-dichloroethyl dimethyl phosphate (technical 85%), Phosdrin® (1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate) technical), Cyanamid 18706 (*O,O*-dimethyl *S*(N-ethyl carbamoyl methyl) phosphorodithioate (technical), Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide) (technical), Bayer 29493 (*O,O*-dimethyl *O*-(4-methylthio-*m*-tolyl) phosphorothioate) (technical, Dimetilan® (3-methyl-5-pyrazolyl dimethyl-carbamate) (technical), Dipterex® (dimethyl 2,2,2-trichloro-1-hydroxyethyl phosphonate) (technical 99%), Delnav® (2,3,-*p*-dioxanedithiol *S,S*-bis(*O,O*-diethyl phosphorodithioate) (technical), DMBC (2,4-dimethyl benzyl chrysanthemumate) (technical), Thanite® (isobornyl thiocyanoacetate) (technical) and G-30494 (S-(2,5-dichlorophenylthio) methyl *O,O*-dimethyl phosphorodithioate) (technical).

Results. The topical LD50s and LD95s for 20 insecticides to susceptible (Wilson line) and Diazinon-resistant (A-strain)house flies are presented in table 1, and the calculated 1d–p lines are shown in figures 1 to 5. The regression coefficients are given in table 2.

The resistant strain, which was able to tolerate 87 times as much Diazinon as the susceptible line of flies, showed increased tolerance (3- to 800-fold) to all materials tested, the highest degree of resistance being exhibited toward heptachlor epoxide (809) and heptachlor (468 fold). Resistance to the other materials was: Delnav (89-fold), Thiodan (58), Thanite (34), dicapthon (18), phorate (17), and Systox (-demeton) (12) and G-30494 (*S*-2 (2,5-dichlorophenylthio)methyl *O,O*-dimethyl phosphorodithioate) (12 fold). Lesser degrees of tolerance had been developed to Bayer 29493 (8-fold), Phosdrin (7), Dipterex (7), Dimetilan (6), Am. Cyanamid 18706 (5), DDVP (4), Dibrom (4), barthrin (3), DMBC (3), and dimethoate (3-fold).

Discussion.—In the course of developing resistance to Diazinon, the house fly strain reported in this study has acquired varying degrees of tolerance to at least 37 other insecticides, many of which had never been used in the barn where the strain was collected. With a few of these materials (the cycloalkanes and DDT analogues), however, there is the possibility that some resistance was present in the population prior to the use of Diazinon.

Between the time the first series (Forgash & Hansens 1959) and present series of compounds were tested, the Diazinon resistance of the strain had increased from 38-

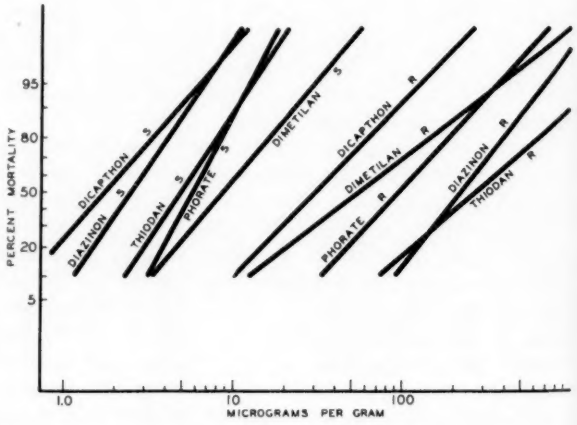

Fig. 1.—Log dosage-probit curves for a Diazinon-resistant strain and a susceptible strain of house fly to Diazinon, dicapthon, Thiodan, phorate, and Dimetilan.

Table 2.—Effect of resistance on the regression coefficient for various insecticides.

COMPOUND	REGRESSION COEFFICIENT		RATIOS OF COEFFICIENTS (RESISTANT/ SUSCEPTIBLE)
	Susceptible Strain	Resistant Strain	
Dipterex	3.00	4.07	1.36
G-30494	2.07	2.57	1.24
Dibrom	3.78	4.34	1.15
Barthrin	3.64	3.99	1.10
Dimethyl benzyl Chrysanthemumate	3.81	3.91	1.00
Dimethoate	3.01	2.97	0.99
Am. Cyanamid 18706	3.85	3.72	0.97
DDVP	4.90	4.70	0.96
Delnav	2.91	2.53	0.87
Diazinon	3.84	3.26	0.85
Dicapthon	2.83	2.35	0.83
Phorate	3.80	2.83	0.74
Phosdrin	4.30	3.09	0.72
Dimetilan	3.08	1.98	0.64
Thiodan	3.92	2.23	0.57
Thanite	5.86	3.01	0.51
Demeton	3.19	1.77	0.56
Bayer 29493	8.18	3.77	0.46
Heptachlor	3.86	0.554	0.14
Heptachlor epoxide	3.88	0.521	0.13

fold to the present level of 87-fold, and thus results of the two tests may not be strictly comparable in all cases. Resistance to ronnel has increased substantially since the initial tests, and although one might suspect that this condition holds true for phosphorothioates in general, there is no assurance. With certain other materials, *i.e.*, (5(1-isopropyl-3-methylpyrazolyl) dimethylcarbamate) pyrethrins, malathion, and Isolan®, there has been no further change in tolerance despite the continual increase in resistance to Diazinon (Forgash & Hansens, unpublished), and this fact probably holds true for some materials structurally related to pyrethrins, malathion, and Isolan. Nevertheless, because of the dearth of knowledge concerning the resistance mechanisms, one may not confidently extend these findings to include all analogues.

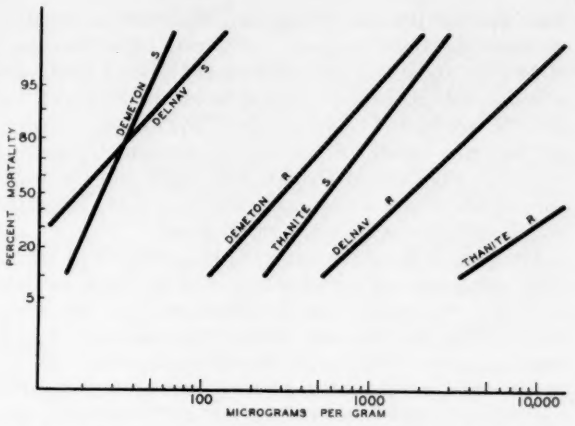

FIG. 3.—Log dosage-probit curves for a Diazinon-resistant strain and a susceptible strain of house fly to demeton, Delnav, and Thanite.

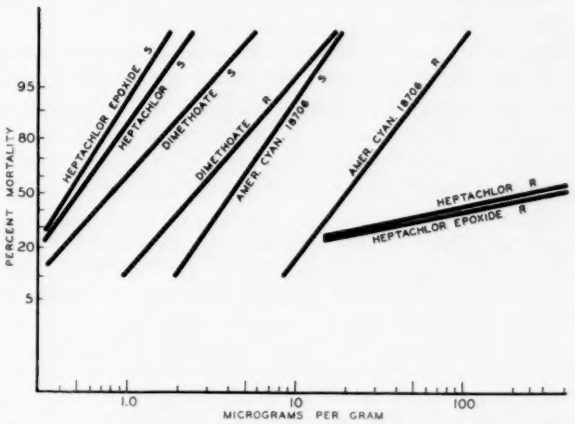

FIG. 4.—Log dosage-probit curves for a Diazinon-resistant strain and a susceptible strain of house fly to heptachlor, heptachlor epoxide, dimethoate, and Am. Cyanamid 18706.

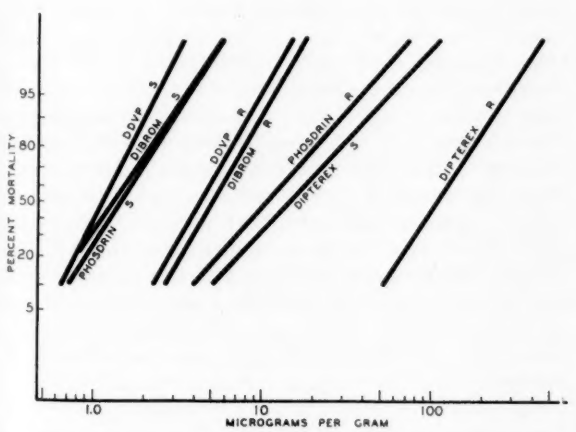

FIG. 2.—Log dosage-probit curves for a Diazinon-resistant strain and a susceptible strain of house fly to DDVP, Dipterex, Phosdrin, and Dibrom.

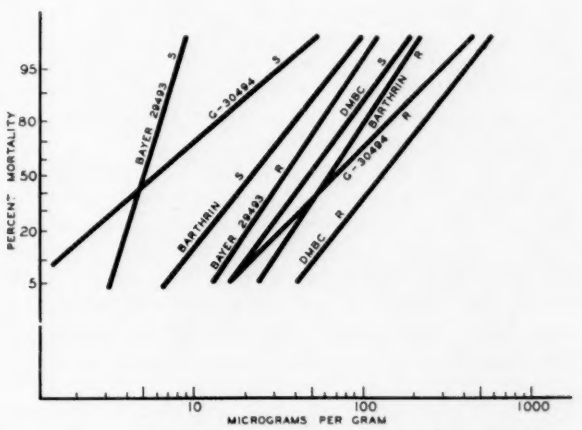

FIG. 5.—Log dosage-probit curves for a Diazinon-resistant strain and a susceptible strain of house fly to barthrin, DMBC, and Bayer 29493, and G-30494.

The results on the phosphorodithioates adequately demonstrate this. As previously mentioned, tolerance (5-fold) to malathion has not changed despite continued selection with Diazinon, and, as might be expected, the level of tolerance to dimethoate is equally low (3-fold). On the other hand, there is a surprisingly high degree of resistance (89-fold) to Delnav, indicating that the tolerance of the strain to this material has steadily increased under continued selection with Diazinon.

The high level of resistance to heptachlor and heptachlor epoxide is not surprising, in view of similar findings for other chlorinated hydrocarbons on this particular strain (Forgash & Hansens 1959). It is noteworthy, however, that the epoxide is the less effective of the two materials since heptachlor apparently exerts toxicity after conversion to the epoxide (Perry *et al.* 1958), and, also, since the epoxide is more toxic than heptachlor to susceptible flies (table 1.). This difference may be due to a penetration effect at high dosages, for the epoxide is less soluble than heptachlor in polar solvents.

With both heptachlor and the epoxide, resistance is accompanied by a sharp decrease in slope of the ld–p line. This condition is similar to previous results with chlordane, dieldrin, and lindane, and may be a manifestation of the relationship between the amount of toxicant applied and that absorbed, and, also, of the variability among individuals in the quantity of insecticide reaching the site of action (Hewlett 1958). This explanation applies equally to other instances of lowered regression coefficients.

There was also appreciable resistance to Thiodan, although the level (58-fold) was the lowest of any found with the eight chlorinated hydrocarbons tested thus far (Dilan excepted).

The increase in tolerance to Dimetilan is the least encountered for any carbamate tested. Previous studies showed resistance levels of 10-, 14-, and more than 37-fold for Pyrolan® (5-(3-methyl-1-phenylpyrazolyl) dimethylcarbamate), Isolan, and Sevin® (1-naphthyl methylcarbamate) respectively (Forgash & Hansens 1959). The most toxic of these four compounds to susceptible, as well as to resistant flies, is Dimetilan, whereas Sevin is the least effective of all; furthermore, the ld–p line of Dimetilan for susceptible flies has the highest regression coefficient of the four carbamates. These correlations agree with the recent suggestion of Moorefield (1960) that the ability to develop resistance to a specific carbamate may be related to the slope of the regression line for that material.

Previous studies have shown that pyrethrins and allethrin are only four to five times as toxic to susceptible flies as to the Diazinon-resistant strain. In the present study it was found that the tolerance of the resistant strain to DMBC and to barthrin has increased but 3-fold, which is as expected, in view of the structural similarity of these materials to allethrin and pyrethrins. Tolerance, in this instance, is probably a vigor type, for studies in progress indicate that the increased ability of this strain to tolerate pyrethrin is not a true physiological resistance.

The high resistance to Thanite (34-fold) is particularly noteworthy, since it was previously found that tolerance to Lethane 384® (2-(2-butoxyethoxy)ethyl thiocyanate) had increased only 2-fold (Forgash & Hansens 1959). It seems unlikely that the latter value has changed with continued selection, but it will be necessary to retest Lethane before attempting to interpret the difference in resistance which appears to exist for thiocyanates.

Resistance to dicapthon is of the order (18-fold) which might have been expected from previous studies (Forgash & Hansens 1959) with the structural analogues, ronnel (18-fold), Chlorthion® (*O*-(3-chloro-4-nitrophenyl) O,*O*-dimethyl phosphorothioate) (23-fold), and parathion (16-fold). The level of tolerance to Bayer 29493, another analogue, was only 8-fold; however, the aryl group of this compound is considerably different from those of the other materials. Between the time that ronnel and Bayer 29493 were tested, the level of resistance to ronnel had nearly doubled, and it is quite possible that resistance to parathion and Chlorthion has changed proportionately, in which case the difference in resistance level between Bayer 29493 and the other analogues is further intensified. Moorefield (1960) has pointed out from a study in a limited number of carbamates that the development of resistance may in some way be related to the homogeniety of response in the fly population to the toxicant, that is, the steeper the ld–p line, the less the tendency toward resistance. In this connection it is noteworthy that the compound to which there was the highest resistance, Chlorthion (23-fold), has the lowest regression coefficient (2.08), and Bayer 29493, to which there is the least increase in tolerance (8-fold), shows the highest regression coefficient.

The low levels of increased tolerance (4- to 7-fold) encountered with DDVP, Dipterex, Dibrom, and Phosdrin indicate that cross-resistance to phosphates and phosphonates in general is less pronounced than to most phosphorothioates and some dithioates.

The great range in resistance (3 to 89-fold) to dithioates is of particular interest, since it shows that the resistance mechanism toward this type of compound is somewhat specific, and recent studies (Forgash & Hansens, unpublished) demonstrated that the tolerance to at least one dithioate (malathion) was not increased further by continued selection with Diazinon. There is no correlation of resistance with either potency or with slope of the ld-p lines for the five phosphorodithioates tested. It should be pointed out that the three most effective materials (dimethoate, Am. Cyanamid 18706, and malathion (Forgash & Hansens 1959) are dimethyl compounds whereas the alkyl groups of both Delnav (89-fold resistance) and phorate (17-fold resistance) are diethyl. The significance of this observation is dubious, however, because of the limited number of materials involved. Moreover, Plapp & Casida (1958a) have demonstrated with five phosphorothioates that alkaline hydrolysis of alkyl groups proceeds more readily with dimethyl than diethyl, and in susceptible flies(Plapp & Casida 1958b), alkyl hydrolysis did not seem to be an important detoxication mechanism against ronnel. Whether this conclusion applies to dithioates and to resistant flies is not known.

The low cross-tolerance to dimethoate and Am. Cyanamid 18706 is rather encouraging, since it suggests that it may be possible to find phosphorodithioates that will give effective control in the wake of Diazinon resistance.

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Effect of Sample Location Within Fields on Corn Earworm and Rice Weevil Infestation and Damage[1]

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ABSTRACT

The analysis of the data from 10 fields showed no significant effect of sample location in relation to field margins on infestation and damage to field corn by the corn earworm, *Heliothis zea* (Boddie), but a highly significant effect on the percentage of ears infested with rice weevil, *Sitophilus oryza* (L.).

In surveys of damage to field corn caused by the corn earworm, *Heliothis zea* (Boddie), one important consideration is the uniformity of infestation or damage within fields. If marginal effects are important, they should be taken into account in such a way as to result in representative sampling. Fields surveyed for corn earworm damage in Georgia in 1957 were sampled schematically to determine the presence or absence of such marginal effect. Infestation by the rice weevil, *Sitophilus oryza* (L.), was recorded at the same time.

Marshall (1936), in examining the distribution of corn earworm eggs at two dates on corn silks in South Africa, found the variation in number of eggs per plant to be very great but distribution was essentially normal within a nearly square block one-eighth acre in size. Variation between rows tended to be greater than within rows but was not consistent. In sampling 1.1-acre plots he found that 16 schematically arranged samples, each containing 1 or 2 plants, were adequate for comparing plots in the same area. Where "variation between different fields affect results, it may be expected to be even more adequate. In fact, some reduction in the percentage of sampling (0.2%) might be advantageous."

In an examination of Marshall's data in the light of continuing development and changing attractiveness of plants to oviposition, Walker (1942) showed that eggs were "not distributed either uniformly or at random among plants" at any one point in time owing to variations in plant development. She concluded that "the actual frequency distribution of the eggs suggests a random as opposed to a uniform distribution but it is shown that the conditions required for a pure mathematical random distribution cannot be satisfied." Wilson (1956), working with fields of insecticide-treated sweet corn in Florida, found earworm egg populations per silk mass and infested ears at harvest to be significantly higher at the margins of fields than at locations halfway to the center and at the center. This could result from treatment effect. Kamel & Johansen (1957) stated that in the State of Washington "infestation was heaviest near the field margins."

PROCEDURE.—Ten fields, selected at random from 98 examined in a limited survey for corn earworm damage, were sampled for corn earworm and rice weevil infestation and damage. It is assumed that none of the fields had received direct insecticidal treatment for control of either insect since this is ordinarily not considered practical on field corn.

Four samples were taken, as shown in figure 1, beginning near the margin and continuing at 25-row or -pace intervals progressively toward the center of each field and at 10-row or -pace intervals to the right or left. The first sample was taken at an easily accessible point near the middle of the side along the road traveled. Each sample consisted of 25 consecutive ears from two adjacent rows. The survey was made in September 1957 when all corn examined was fully mature. These 10 fields were located in two 70-mile-square survey areas in south-central and east-central Georgia. The other 88 fields were sampled only at locations "a" and "b."

Infestation and damage were determined by stripping back the husks, counting the number of weevils present, and measuring the depth of corn earworm damage to the nearest one-fourth inch along the longitudinal axis be-

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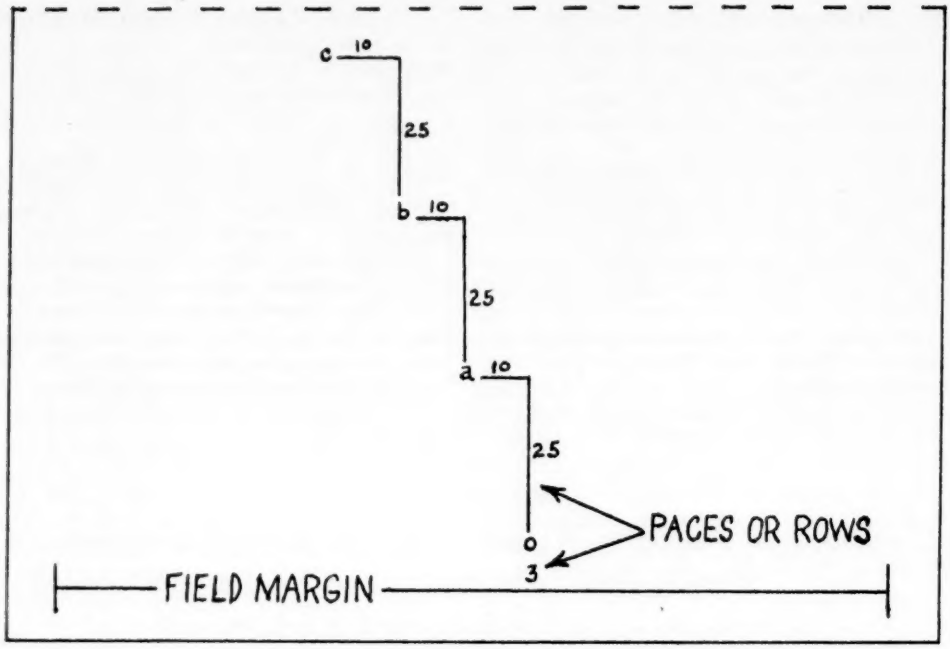

FIG. 1. Sample locations in relation to field margin.

ginning at the tip kernels. Ears were considered as damaged by corn earworm only when kernels had been eaten or damaged. If only rice weevil damage or tunneling was observed, the weevil population was listed as 1.

RESULTS.—The data for four sample locations are summarized in table 1. Only one field showed a consistent trend of corn earworm infestation and damage from high at the margin to low toward the center, and none consistently showed the reverse. The average percentage of damaged ears and the depth of penetration of damaged kernels on the ear were less at the field margin than at the other locations. Average ear length was also slightly less at the margin. No significant difference was found between locations within fields for any of these three characteristics. Fields differed significantly at the 1% level in depth of penetration but not in percentage of ears damaged.

Rice weevil data showed significant difference at the 1% level for percentage of ears infested and at the 5% level for average number per ear, both among locations and among fields. Both measurements showed much greater infestations at field margins and a regular decrease toward the center.

Similar data for all 98 fields in which samples were taken at locations "a" and "b" are summarized in table 2. No difference in earworm infestation or damage was found between the two locations. The average percentage of ears infested by rice weevil was higher at location "a" than at location "b." However, the average number of weevils per ear differed only slightly. This was caused largely by one field of an early loose-husked variety having all ears heavily infested. If this one field is eliminated, the data, 2.4 and 2.0 weevils per ear for locations "a" and "b," respectively, show the trend obtained in the 10 selected fields.

Although weevil infestations were generally and significantly higher nearest field margins, the data were variable. Data for samples "a" and "b" from 98 fields are summarized in this respect in table 3. In slightly over 40% of the fields, location "b" showed infestations equal to or greater than location"a."

In general, data obtained from 2 locations in 98 fields substantiated that obtained from 4 locations in 10 fields in that no effect was found between sample locations within

Table 1.—Effect of sample location in 10 corn fields on corn earworm and rice weevil infestation. Georgia, 1957.

Location Within Fields	Ear Length (In.)	Corn Earworm		Rice Weevil	
		Ears Damaged (%)	Depth Penetration (In.)	Ears Infested (%)	Number per Ear
o	5.29	78.8	0.79	36.8	3.66
a	5.44	82.4	.83	23.2	1.50
b	5.53	82.0	.95	19.2	1.18
c	5.44	84.4	.86	9.6	.66
Average	5.42	81.9	.87	22.2	1.75
F value:					
Locations	.09	.15	.44	5.14**	4.05*
Fields	1.20	2.10	5.72**	5.67**	3.78*

Table 2.—Comparison of paired sample locations in 98 fields surveyed for corn earworm and rice weevil infestation. Georgia, 1957.

Location Within Fields	Corn Earworm		Rice Weevil	
	Ears Damaged (%)	Depth Penetration (In.)	Ears Infested (%)	Number per Ear
a	73.1	0.72	30.5	3.6
b	73.8	.72	24.9	3.5
Average	73.5	.72	27.8	3.5

Table 3.—Distribution of fields by relative degree of sample infestation by rice weevil. Georgia, 1957.

Infestation	Infested Ears		Weevils per Ear	
	Number of Fields	Per Cent	Number of Fields	Per Cent
Higher at location a	54	55.1	53	54.1
Higher at location b	31	31.6	39	39.7
Equal at both locations	10	10.2	3	3.1
Not infested	3	3.1	3	3.1

fields for corn earworm, and significant differences were found for rice weevil infestations.

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Field Experiments Against Several Late-Season Cotton Insects in 1959[1]

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Abstract

Strobane-DDT was as effective against the boll weevil (*Anthonomus grandis* Boh.) and the bollworm (*Heliothis zea* (Boddie)) as toxaphene-DDT. Guthion-DDT gave better control of the boll weevil than Shell SD-3562 and Shell SD-4402 but was no better than Shell SD-5539-DDT. Sevin gave better boll weevil control than Geigy G-30494-DDT but it was not better than Bayer 25141. There was no difference in boll weevil and bollworm control obtained with Sevin applied in a dust or in a wettable powder formulation. Shell SD-4402 was effective against bollworms but Shell SD-3562 was ineffective. Bayer 25141 at dosages used was less effective than standard materials. There was no difference in control of the cotton aphid (*Aphis gossypii* Glov.) and the desert spider mite (*Tetranychus desertorum* Banks) with demeton, ethion, Trithion, Bayer 25141, Shell SD-3562 and Geigy G-30494. Sevin, Shell SD-3562, and Shell SD-4402 were as effective as methyl parathion against the cotton leafworm (*Alabama argillacea* (Hbn.)). *Bacillus thuringiensis* showed some promise in the control of this insect.

Insecticides with trade marked designations mentioned above are: Bayer 25141 (*O,O*-diethyl *O*-(*p*-methylsulfinyl) phenyl phosphorothioate), Geigy G-30494 (*S*-(2,5-dichlorophenylthio) methyl *O,O*-dimethyl phosphorodithioate), Guthion® (*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3-(4*H*)-ylmethyl)phosphorodithioate), Sevin® (1-naphthyl methylcarbamate), Shell SD-4402 (1,3,4,5,6,7,8,8-Octachloro-3a,4,7,7a-tetrahydro-4,7-methanophthalan), Shell SD-3562 (dimethyl 1-(dimethylcarbamoyl)-1-propen-2-yl phosphate), Shell SD-5539 (m-Nitrobenzyl 3-(dimethoxyphosphinyloxy) crotonate), Strobane® (a mixture of chlorinated terpenes with about 66% of chlorine), and Trithion® (*S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate).

Heavy late-season infestations of several cotton insects in central Texas in the vicinity of Waco in 1959 afforded an opportunity for an evaluation of several new insecticides in the field. The predominating species were the boll weevil (*Anthonomus grandis* Boh.) and the bollworm (*Heliothis zea* (Boddie)). The cotton aphid (*Aphis gossypii* Glov.), the desert spider mite (*Tetranychus desertorum* Banks), and the cotton leafworm (*Alabama argillacea* (Hbn.)) were of less importance.

PROCEDURE.—In the experiments conducted for control of the boll weevil, bollworm, cotton aphid, and desert spider mite the plots were 16 rows wide and of sufficient length to make 0.2 acre. They were arranged in randomized blocks with four replications of treatments. In the cotton aphid and spider mite experiment, untreated plots were not included within the experimental design, but were located in the same field adjacent to the treated plots. In the cotton leafworm experiment the plots were 16 rows wide and 0.5 acre in size and the treatments were not replicated.

Methods of applying insecticides were the same as those previously reported (Cowan *et al.* 1956). In the boll weevil-bollworm experiments the first two or three applications were made at 5-day intervals, and the remainder of the applications at 3- to 4-day intervals. Only one application was made in the cotton aphid, spider mite, and cotton leafworm experiments.

The procedures for recording data were the same as those reported by Ewing & Parencia (1943), Parencia *et al.* (1954), and Cowan *et al.* (1958).

CONTROL OF BOLL WEEVILS AND BOLLWORMS (TABLE 1).—In experiment 1 the boll weevil infestation was severe. A 13-day delay between the third and fourth applications because of rain made conditions for obtaining control very difficult. Each insecticide reduced the infestation significantly below that of the check, and toxaphene-DDT spray was better than Strobane (a mixture of chlorinated terpenes with about 66% of chlorine) plus sulfur dust. There was no difference in bollworm control or yield between insecticides.

Experiment 2 was conducted in the same field and under the same conditions as experiment 1. DDT was not included with Shell SD-3562 (dimethyl 1-(dimethylcarbamoyl)-1-propen-2-yl phosphate) until the fourth appli-

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[2] In cooperation with the Texas Agricultural Experiment Station.

Table 1.—Effectiveness of several insecticides applied as dusts and sprays against the boll weevil and bollworm in field-plot experiments.

Insecticide[a]	Active Ingredient (lb./a.)	Weevil Punctured Squares (%)	Bollworm-Injured Squares (%)	Bollworm-Injured Bolls (%)	Seed Cotton (lb./a.) Total	Seed Cotton (lb./a.) Gain Over Check
Experiment 1 (8 applications between July 7 and August 14)						
Dusts:						
Toxaphene 20+sulfur 30	3.7+5.6	53.0	2.5	3.7	1,126	632
Strobane 20+sulfur 30	3.5+5.2	55.7	1.8	3.6	1,142	648
Sprays:						
Toxaphene 4+DDT 2	2.4+1.2	47.7	1.8	3.6	1,084	590
Strobane 4+DDT 2	2.4+1.2	51.1	2.1	4.3	1,092	598
Methyl parathion 2+DDT 2	0.5+1.2	50.7	2.1	5.1	1,020	526
Untreated	—	75.3	4.1	8.0	494	—
L.S.D. at 5% level	—	6.2	0.9	1.7	206	—
Experiment 2 (9 spray applications between July 7 and August 19)						
Shell SD-5539, 2+DDT 2	0.59+1.17	42.7	2.2	5.5	1,027	623
Shell SD-3562, 10+DDT 2[b]	1.08+1.25	49.8	3.8	11.0	717	313
Shell SD-4402, 1.25	0.59	54.9	2.7	4.7	1,055	651
Guthion 1.5+DDT 2	0.29+1.17	36.5	2.4	4.8	1,039	635
Untreated	—	73.5	6.6	12.8	404	—
L.S.D. at 5% level	—	9.7	1.2	2.9	180	—
Experiment 3 (7 applications between July 10 and August 12)						
Dust:						
Sevin 10+sulfur 40	1.9 +7.6	55.5	2.0	2.2	1,624	484
Sprays:						
Sevin[c]	1.79	53.4	1.7	2.3	1,593	453
Geigy G-30494, 2+DDT 2	0.64+1.0	60.3	2.5	2.8	1,624	484
Bayer 25141, 2	.64	55.2	2.8	5.1	1,418	278
Malathion 5+DDT 2	1.68+1.0	60.9	1.9	2.5	1,564	424
Untreated	—	88.5	5.4	6.3	1,140	—
L.S.D. at 5% level	—	4.5	2.3	2.3	251	—

[a] Pounds per gallon in sprays, per cent in dusts.
[b] DDT added in last six applications.
[c] Wettable powder, 85% active ingredient.

cation, when it became apparent that this material alone would not control the bollworm. Each insecticide reduced the boll weevil infestation significantly below that of the check, and Guthion® (*O,O*-dimethyl *S*-(4-oxo-1,2,3,-benzotriazin-3-(4*H*)-ylmethyl) phosphorodithioate) plus DDT was better than any of the other insecticides except Shell SD-5539 (m-Nitrobenzyl 3-(dimethoxyphosphinyl-oxy) crotonate) plus DDT. Guthion-DDT, Shell SD-5539-DDT, and Shell SD-4402 (1,3,4,5,6,7,8,8-Octachloro-3a,4,7,7a-tetrahydro-4,7-methanophthalan) gave significantly better bollworm control and higher yields than Shell SD-3562. Although the yield with the latter material was better than in the check, DDT was added to it too late to offset bollworm damage.

In experiment 3 the boll weevil infestation was also severe. Sixteen days elapsed between the second and third applications because of rainy weather. In an effort to regain control, the third and fourth applications were made at 3-day intervals. Each insecticide reduced the boll weevil infestation significantly below that of the check. Sevin (1-naphthyl methylcarbamate) applied in either a wettable-powder spray or dust formulation, and Bayer 25141 (*O,O*-diethyl *O*-(*p*-methylsulfinyl) phenyl phosphorothioate) in a spray were better than Geigy G-30494 (*S*-(2,5-dichlorophenylthio) methyl *O,O*-dimethyl phosphorodithioate) plus DDT, and malathion

Table 2.—Effectiveness of several insecticides against the cotton aphid.

Insecticide (lb./gal.)	Active Ingredient (lb./a.)	No. of Aphids per Leaf: Before Treatment	After Treatment on Days Shown: 1	4	8	Average
Experiment 1 (1 application August 6)						
Demeton 2	0.25	101.5	0	0	0	0
Ethion 4	1.0	101.8	0.30	0	0	0.10
Trithion 4	1.0	88.7	0	0	0	0
Bayer 25141, 2	0.25	85.7	0.22	0	0	0.07
Shell SD-3562, 10	.5	103.9	0.04	0	0	0.01
Geigy G-30494, 2	.25	98.7	0.05	0.04	0	0.03
L.S.D. at 5% level	—	—	—	—	—	—
Untreated (outside check)	—	92.6	61.4	33.9	3.5	32.9

Table 3.—Effectiveness of several acaricides against the desert spider mite.

Acaricide (lb./gal.)	Active Ingredient (lb./a.)	No. of Spider Mites per Leaf: Before Treatment	After Treatment on Days Shown: 1	4	8	Average
Experiment 1 (1 application August 6)						
Demeton 2	0.25	12.7	0	0	0	0
Ethion 4	1.0	15.7	0	0	0	0
Trithion 4	1.0	13.1	0	0.01	0	0.003
Bayer 25141, 2	0.25	12.9	0	0.09	0	0.03
Shell SD-3562, 10	.5	19.4	0	0.03	0	0.01
Geigy G-30494, 2	.25	13.5	0	0.04	0	0.01
L.S.D. at 5% level	—	—	—	—	—	—
Untreated (outside check)	—	14.0	7.1	9.4	12.0	9.5

Table 4.—Effectiveness of several insecticides against the cotton leafworm.

Insecticide (lb. per gal.)	Active Ingredient (lb./a.)	No. of Larvae per Plant					
		Before Treatment	After Treatment on Days Shown				
			1	2	3	5	7
Experiment 1 (1 application on August 19)							
Bacillus thuringiensis[a]	1.0	17.4	17.7	11.6	8.4	3.4	4.5
Toxaphene 4+DDT 2	2.0+1.0	16.0	4.3	2.5	—	1.4	—
Shell SD-3562, 10	0.25	18.6	0.1	0	—	—	—
Shell SD-4402, 1.25	.5	14.3	1.0	0.2	—	—	—
Methyl parathion 2	.25	17.8	0	0	—	—	—
Sevin[b]	1.0	19.0	0	0	—	—	—

[a] Wettable powder containing 70 billion spores per gram.
[b] 85% wettable powder.

plus DDT applied in sprays. All the other insecticides gave better bollworm control than Bayer 25141. There was no difference between insecticides in yield, but the lowest increase was produced by Bayer 25141, reflecting the poorer bollworm control.

CONTROL OF THE COTTON APHID AND A SPIDER MITE (TABLES 2 AND 3).—There was no significant difference in the control of the cotton aphid and desert spider mite 1, 4, and 8 days after treatment between demeton, ethion, Trithion® (*S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate), Bayer 25141, Shell SD-3562, and Geigy G-30494.

CONTROL OF COTTON LEAFWORMS (TABLE 4)—Good kills of cotton leafworms were obtained 1 day after treatment with Shell SD-3562, methyl parathion and Sevin. Shell SD-4402 was somewhat slower in action but reductions obtained with it were good also. Toxaphene-DDT gave only fair control. The kill obtained with *Bacillus thuringiensis* was slow, but it reduced the population by 74% seven days after treatment.

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Field Methods to Determine the Infestation of the Sorghum Webworm and the Damage by the Sorghum Midge in Grain Sorghum[1]

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ABSTRACT

Techniques for sampling sorghum webworm, *Celama sorghiella* (Riley), infestations and sorghum midge, *Contarinia sorghicola* (Coq.), damage in four varieties of grain sorghum were evaluated. No significant correlation occurred between field estimates and standard counts (actual number) of webworm in two tight-headed varieties, Combine Kafir 60 and Martin. Significant correlation did exist between most field and standard webworm counts in case of the open-headed varieties, DeKalb E56a and RS 610. A small range of percentage for rating the degree of midge damage to field grain sorghum had a higher significant correlation to actual grain yields than resulted from a rating method using a larger range.

Acreage devoted to the production of grain sorghum in Texas has increased greatly during the past 10 years and more serious insect problems have arisen. Two insects of economic importance on grain sorghum are the sorghum webworm (*Celama sorghiella* (Riley)) and the sorghum midge (*Contarinia sorghicola* (Coq.)). The sorghum webworm is a sluggish caterpillar that is particularly prevalent during wet seasons. The worms devour the seed and may completely destroy the grain of sorghum heads. The number of worms in a head may range from one to as many as a thousand. Sorghum midges are small (2 mm. long) red flies which as larvae develop inside the glume where it extracts plant juices from the seed of sorghum or related plants. Larval feeding causes the seed to blast.

This experiment was designed to evaluate different methods for taking infestation records and for determining insect damage to grain sorghum. One phase of this experiment involved the use of several test methods to determine the intensity of field infestations of the sorghum webworm in four varieties of grain sorghum. Another part of the experiment concerned a comparison of two rating methods to estimate the damage caused to grain sorghum by the sorghum midge. Data obtained by these methods were evaluated on the basis of the actual grain harvested from each grain sorghum plot in the experiment.

EXPERIMENTAL PROCEDURE.—It is impractical to obtain actual infestations of the sorghum webworm in grain sorghum. This experiment involved a study of the use of several field-sampling methods to estimate the size of the webworm populations. The sampling techniques tested were included as part of an insecticide performance test which included seven treatments, each of which was replicated four times. Treatments were applied to 1/10-acre plots which were divided into four equal subplots planted with each of four varieties of grain sorghum. Varieties RS 610, DeKalb E56a, Combine Kafir 60, and Martin were used.

The object of this study was to find the least number of

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plants requiring examination to obtain an adequate estimate of the infestation of webworms in the area. Seven field methods, differing only in the number of plants examined, were compared with a standard. Field records for each of the seven methods under study were obtained by counting the webworms found in 5, 10, 15, 20, 25, 30, and 35 consecutive heads in each of the subplots. Examinations were made in the following manner: each sorghum head was parted longitudinally on one side by use of hands and then the head was twisted sidewise and similarly opened on the other side and the exposed worms up to a maximum of 10 were counted. An entry of "10+" was made to identify infestations greater than 10 worms per head. The seven field counts were recorded as worms per head and were correlated with the number of worms found by a "standard" technique. Data used as a standard were obtained by counting the worms found in five sorghum heads selected at random within each subplot. Sorghum head samples were placed in paper bags and taken to a laboratory for examination of webworms. Individual heads were removed from each paper bag and "beaten" on $\frac{1}{4}$-inch mesh hardware cloth to dislodge the worms into a pan below the screen. The number of worms per head was recorded.

This experiment also included the evaluation of two rating systems, methods A and B, table 1, devised to estimate sorghum midge damage to grain sorghum. These two methods were compared with a standard method (method C, table 1) which was based on yields of sorghum grain. Method A was formulated prior to this experiment by state and federal workers engaged in cereal and forage insect studies. Damage ratings were based on the percentage of sorghum heads blasted by midges. A number was assigned to each category that corresponded to the estimated per cent damage observed for each of the two rating systems. Method A was based upon the calculated damage by the midge to 10 sorghum heads examined consecutively in each split-plot while method B was based upon examination of 25 heads. The sum of each rating of midge damage to sorghum heads in each plot was correlated to the yields and also methods A and B were correlated, table 1. Damage to varieties were not considered independently.

Table 1.—Comparing two methods of rating sorghum midge damage to yields of sorghum grain.

Methods	Methods Compared	Numbers of Pairs of Observations	Degrees of Freedom	Sample Value of Correlation Coefficient
A[a]	A:B	112	110	0.5018
B[b]	A:C	112	110	.3764
C[c]	B:C	112	110	.6545
r values required for significance			19.1 = .186	
			99.1 = .243	

[a] Method A: based on ratings of sorghum midge damage to 10 heads per observation using the values of the following numbers:

1 = no damage
2 = less than 10% damage
3 = 10% to 25% damage
4 = over 25% damage

[b] Method B: based on ratings of sorghum midge damage to 25 heads per observation using the values of the following numbers:

1 = no damage
2 = 1% to 10% damage
3 = 11% to 25% damage
4 = 26% to 50% damage
5 = 51% to 90% damage
6 = 91% to 100% damage

[c] Method C: based on yield of sorghum grain in lbs./acre.

Table 2.—Correlation coefficients of different methods of taking field infestations of sorghum webworms to actual populations on four varieties of grain sorghum.

Methods	Number of Heads	Sample Value of Correlation Coefficient on: Combine Kafir 60	Martin	DeKalb E56a	RS 610
1	5	0.21[a]	0.06[a]	0.41*	0.43*
2	10	.03[a]	.27[a]	.48**	.48**
3	15	.09[a]	.25[a]	.52**	.49**
4	20	.35[a]	.09[a]	.28[a]	.39*
5	25	.06[a]	.16[a]	.47*	.50**
6	30	.28[a]	.02[a]	.37[a]	.47*
7	35	.28[a]	.01[a]	.44*	.49**
Standard	5				
r values required for significance			19.1 = .374		
			99.1 = .478		

[a] Negative correlations.
* Correlation significant at 5% level.
** Correlation significant at 1% level.

RESULTS.—Varieties of grain sorghum, Combine Kafir 60, and Martin have very compact heads; RS 610 is a moderately open-headed type, and DeKalb E56a is an open-headed variety. No significant correlation existed between field estimates and standard counts of webworm infestation in the two tight-headed varieties, table 2. Significant correlation existed between most field and standard webworm counts in both DeKalb E56a and RS 610 varieties, table 2. Only field counts of webworms found in 20 and 30 heads of the variety DeKalb E56a had no significant correlation to the standard.

The lack of correlation between types of sorghum having characteristically tight heads was probably owing to the technique used for estimation of the number of worms present. Compact heads opened longitudinally had relatively less internal area exposed than did open-headed type varieties. Since RS 610 and DeKalb E56a are the popular commercial varieties at this time, the sampling methods analyzed in this experiment may be satisfactorily used in the field. The examination of as few as five heads in approximately 30 different areas in large fields may be ample for an accurate estimate of the population of webworms in sorghum. The field methods used for estimations of the number of worms per head in variety RS 610 and DeKalb E56a resulted in an approximate ratio of one worm per head for field counts to 4 to 5 worms per head in actual counts. An infestation of 1 to 5 sorghum webworms per head apparently is sufficient to warrant chemical control and produce economical grain yields. Stage of growth and potential yield of grain sorghum are factors that may determine the feasibility of the application of insecticides for the control of this insect.

Results indicate that of the two methods of rating sorghum midge damage method B has a higher significant correlation to yields; therefore, method B may be the preferred system. A practical method of estimating damage to grain sorghum by the midge during early season may be helpful in predicting heavy infestations in late maturing sorghum.

Gypsy Moth (*Porthetria dispar*) Detection with the Natural Female Sex Lure[1]

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Abstract

Traps baited with the sex lure secreted by the nonflying gypsy moth (*Porthetria dispar* (L.)) female attract and ensnare the flying male of the species. With these traps large areas may be scouted for the presence of the moth. Control measures are applied to fringe areas of the infestations where the moth is found to prevent further spread of the insect.

Trap design, trap color, bait dispensers, duration of bait effectiveness, bait strength requirements, trap height, trap placement, collection of the sex lure, and other investigations related to the gypsy moth trapping program are discussed.

Of late there has been an upsurge in interest in attractants as aids in insect control. The gypsy moth control program conducted by this Department was the first to use an attractant on a large scale for detection, traps baited with the sex attractant extracted from the nonflying females being deployed over large areas for this purpose. In responding to the lure, the flying males betrayed their presence, thereby pinpointing the areas needing treatment. The use of the traps in the fringe areas of the infestation and the follow-up control measures, usually DDT sprayings by airplane, have prevented the spread of the moth from the Northeastern part of the United States to neighboring areas.

Attempts to use traps baited with living virgin females for control were unsuccessful although many males were caught (Forbush & Fernald 1896). Later the same traps were found to be efficient detectors of moth infestations. Major advances were the findings that living females could be replaced by their last two abdominal segments and that an extract of these tips was likewise effective (Collins & Potts 1932); but the tips, or their extract, deteriorated over a period of time, so that lure collected one summer was much less effective during the flight season a year later. However, Haller *et al.* (1944) were able to stabilize the lure by a chemical processing (hydrogenation). Recently, Acree *et al.* (1959) reported that extracts processed in this manner, when stored at 40° F., lost practically none of their attractiveness over a 10-year period.

With a means of preparing a lasting lure available, scientists in the Department of Agriculture worked out the many details necessary for the successful operation of the survey program and have continued their attempts to improve trapping efficiency. This paper presents the most recent investigations, which have led to the currently used procedure.

Studies on Traps.—*Modified Graham Trap.*—In current use in gypsy moth trapping surveys is the modified Graham trap shown in figure 1. It is a hollow metal cylinder about 7 inches long and 4 inches in diameter, equipped with removable rings that hold at each of the open ends a lightly waxed paper cone with a 1-inch diameter hole at its apex. Moths that are enticed into the hole are prevented from escaping when they get stuck on an adhesive-covered waxed-paper liner placed inside the trap. The trap is baited with a crepe filter-paper dispenser charged with the female sex lure. The dispenser is held to the top of the trap by means of a wire that also fastens the trap to a tree. Care must be taken not to extend the adhesive to the edge of the paper liner, as contamination of the dispenser with adhesive greatly reduces the activity of the sex lure.

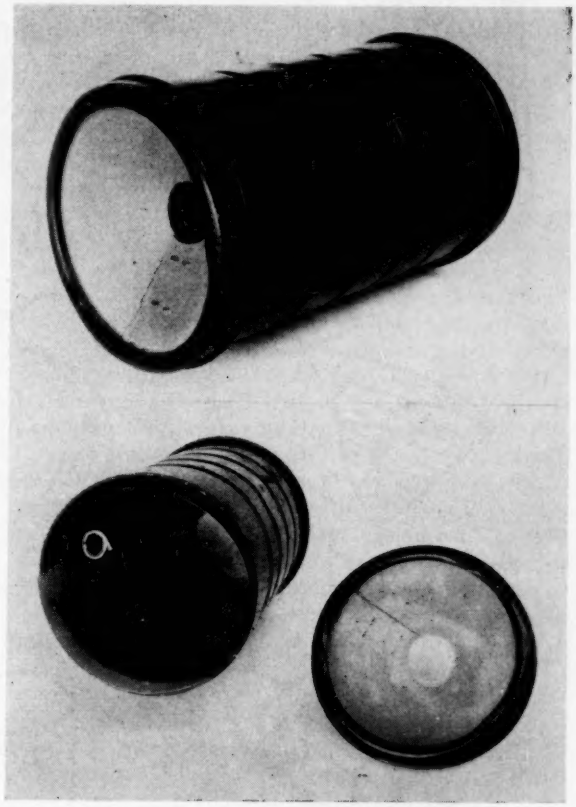

Fig. 1.—Modified Graham trap.

Trap Design.—At first a cage containing living females was nailed to a tree trunk and surrounded by a sticky substance that served to catch males coming to the traps (Collins & Potts 1932). When living females were replaced by tip extracts, the trap was modified. One such modification (fig. 2a) was an adhesive-covered waxed paper in the center of which was placed an inverted waterproof paper cone containing the lure on a cotton wad. Some other traps that were tested are shown in figure 2, b to j, the best of these being designed by S. F. Potts (fig. 2b) and Frank Graham (fig. 2j) of this Department. A smaller "soup-can-size" version of the modified Graham trap, a trap with four entrances at 90° to each

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[2] Retired.

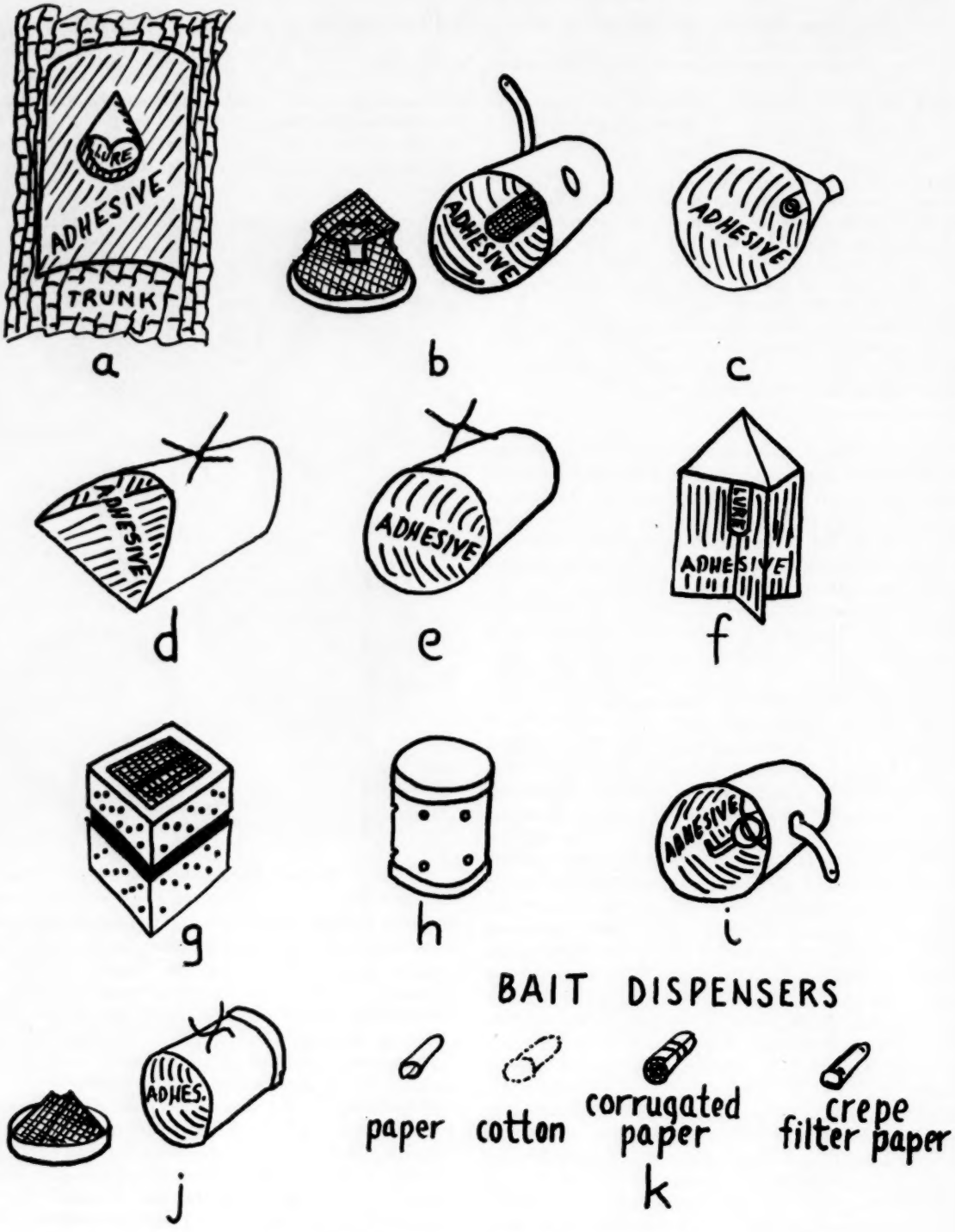

FIG. 2.—Experimental trap designs and bait dispensers.

other, and an awning-shaped trap were also tested.

The original Graham trap (fig. 2j) was identical with the modified version (fig. 1) except that it had wire-screen ends. It is believed that the paper ends improved trap performance by maintaining the adhesive in a tacky condition longer and by offering to the insect a smoother entrance hole. Variations in the trap entrance did affect moth captures; in one experiment average captures (4 replicates) of the original trap were elevated from 58 to 82 by soldering the wire-screen ends to give a smooth entrance for the insect.

Size of trap openings is important. Although more

moths can enter a trap with a larger opening, they can also escape more easily. The amount of lure volatilized also depends on the size of the opening. The 1-inch opening of the Graham trap gave the best results. A short wire placed across the entrance to prevent birds from becoming caught reduced moth captures to less than half. Other attempts to improve performance, such as roughening the paper around the trap entrance and using a smooth grommet-lined entrance, offered little or no increase in trap efficiency.

The only trap that outcaught the original Graham, and then mainly during heavy moth flight, was the Potts trap (fig. 2b), probably because of its greater size (12″×6″ diameter) and consequently much greater area of adhesive-covered liner. Early in the season and again towards the end of flight when moths were few in number, captures scarcely exceeded those obtained with the Graham trap. Hence, in critical areas—those with low moth populations—the two may be expected to perform equally well. The Graham trap, being smaller, less complicated, and less costly than the Potts trap, was therefore preferred.

The position of the bait dispenser in the trap was varied but placement horizontally at the roof, at the base, or centrally, had little effect on the number of moths caught. Fastening the cartridge by means of a wire through two holes in the roof was adopted as the most convenient arrangement.

Effect of Trap Color.—To determine whether the male gypsy moth has any color preference, traps of different colors were tested. Statistical analysis of the moth captures in initial tests with gray, red, yellow, white, and green traps showed no significant differences, although the yellow, green, and gray traps were the most effective. Further tests with green, gray, yellow, yellow-orange, and orange confirmed the previous finding that trap color is of little importance in trapping work. Gypsy moth traps in current use are painted dark green in order that they may blend with surrounding foliage and thus be protected from vandalism or theft.

Bait Dispensers (Fig. 2k).—The first bait dispenser used in survey work was a 5-gram roll of absorbent cotton capable of holding about 30 ml. of attractant material dissolved in benzene. This dispenser was later replaced by one made from a sheet of corrugated paper rolled up to form a cylinder 3″ long and 1″ in diameter and held together with gummed-paper tape. Only 2.5 ml. of benzene is required to saturate this dispenser. The following experimental data are pertinent:

Dispenser	*Average Catch (4 Replicates)*
Cotton	22.0
3″×1″ Corrugated paper	44.5
1″×1″ do.	36.3
3″×½″ do.	33.3
1″×½″ do.	27.3

The difference between the various sizes of corrugated-paper dispensers did not reach the level of significance (least significant (5%) mean difference 20.29), but there was a significant difference favoring the 3″ by 1″ corrugated dispenser over the cotton wad.

Corrugated-paper dispensers were used between 1948 and 1955, but it was then discovered that the paper may contain impurities that interfere with the activity of the attractant; some samples were found to reduce gypsy moth captures as much as 50%. Chemical analysis of several grades of corrugated paper at the University of Massachusetts showed the presence of acid residues. To remedy this situation several types of filter paper known to be available in uniform and constant quality were tested and one was found to be highly satisfactory. It was crepe filter manufactured by Eaton Dikeman Co., No. 634. The dispenser was made by rolling a 3″ by 4″ sheet into a cylinder 3″ long and ½″ in diameter and stapling to hold it in cylindrical form. Four ml. of benzene is required to saturate this cartridge.

In tests conducted in 1955 at more than 50 sites representing a wide variety of field conditons, the filter-paper dispensers caught a total of 4,009 moths while the corrugated dispenser caught 4,420 moths. Neither type of dispenser consistently outcaught the other, and most noteworthy was the fact that, although the corrugated dispensers outcaught the others on the early patrols, the reverse was true on the last patrols. This result dispels any belief that filter-paper bait dispensers might lose their effectiveness in late season. Furthermore, at those sites where moth captures were small (less than 10 per trap), the catches obtained with the filter paper were greater than those with the corrugated dispensers. This result has been given considerable weight inasmuch as the most important traps in the field survey are those placed in areas where the gypsy moth population is sparse.

Mold appeared on most of the filter-paper dispensers during the test season, but there was no indication that it impaired their attracting power. The corrugated dispensers did not develop mold in these tests.

Adhesives and Their Duration of Effectiveness.—Although the gypsy moth sex lure remains effective for a full season, the adhesive (Tanglefoot[3]) should be "freshened" by combing with a serrated paddle at least once a week. The surface of the Tanglefoot has a tendency to harden or glaze during cool nights or over a period of days and, even though apparently "tacky" to the touch, it is sufficiently altered from its original condition to permit moths to escape. Combing restores an effectively tacky surface. Additional adhesive is added as necessary, particularly after heavy catches.

The results obtained with 16-tip traps set out in duplicate in each of four replicates show that the more frequent combing increases the catching efficiency of the trap as indicated here:

Frequency of Combing	*Average Catch (4 Replicates)*
3 Days	93.8
	76.3
6 Days	63.0
	42.5
9 Days	34.8
	38.8
Uncombed all season	12.8
	15.5

Catches in the 6- and 9-day traps declined toward the end of the untended periods. Traps uncombed throughout the season made very low total catches, and there

[3] Manufactured by The Tanglefoot Company, Grand Rapids, Michigan.

were practically no captures after 3 weeks. All traps were combed just prior to the start of the moth flight.

It is impractical to visit traps in the field as often as every 3 days, but an interval of 6 to 9 days between combings, preferably 6, gives reasonable trap efficiency.

A great many samples of adhesives were investigated as a substitute for Tanglefoot but even the best of these caught about the same number of moths as Tanglefoot and showed no superiority in the need for frequent combing.

Deactivation of Lure by Adhesive.—The gypsy moth sex lure is effective in the presence of the Tanglefoot adhesive as long as there is no direct physical contact between the two. Bait atomized on Tanglefoot attracted no moths. Since contamination of dispensers with adhesive by accidental rubbing against adhesive-covered liners (especially while servicing traps) might have an adverse effect on catching power of the lure, a line of traps containing dispensers contaminated in several ways was set out; it gave the following results:

Dispenser Contamination	*Average Catch (4 Replicates)*
None	52.8
Sides slightly smeared	33.3
One end smeared	0.5
Sides completely smeared	3.3
Entire cartridge smeared	0.3
Both ends smeared	4.0

Contamination on the sides of the dispenser was detrimental, but at the ends it practically inactivated the lure. Hence greatest care should be exercised to avoid contamination of the dispensers with adhesive, especially while servicing traps.

Salvaged Cartridges.—The effectiveness of the hydrogenated sex lure at the end of a season's exposure prompted consideration of the possibility that cartridges exposed for one season might be usable the next year. However, salvaged cartridges, when used singly, caught not more than 25% as many moths as those freshly prepared. Doubling and tripling salvaged cartridges did reduce the incidence of "duds," but the performance failed to match that of the fresh product. Addition of benzene alone added no strength. The addition of fresh lure was beneficial but not consistently so. The latter procedure may be worthy of consideration if supplies of bait material become inadequate, particularly for less important phases of the trapping program.

In another attempt to utilize salvaged lure, the attractant was extracted from the cartridges at the end of a season's exposure and tested the following year on a fresh cartridge. This procedure eliminated "duds" by pooling all the lure, but it likewise failed to bring performance up to standard. Performance was especially poor in areas of low infestation.

Although corrugated dispensers did not normally mold during their first season in the field, a greenish mold was present on some salvaged dispensers returned at the end of the 1950 trapping season. In tests the next summer the dispensers were found to be appreciably less active than the others.

TRAP PLACEMENT.—*Height.*—In the late 19th century it was determined that the response of male gypsy moths is greatest to caged females that are located close to the ground. One of our experiments to determine the effect of trap height is summarized below:

Feet Above Ground	*Average Catch (4 Replicates)*
0	27.0
3	22.0
6	34.5
12	15.5

There is thus little difference in trap captures between ground level and 6 feet above it. In survey work traps are usually fastened to a tree about 3 or 4 feet above ground.

Placement in Scouting Surveys.—Many tests were carried out to determine the best method of trap placement, since some traps have failed to attract moths, even with fairly sizable infestations nearby. Obviously the best trap will be of little value if natural conditions interfere with its proper functioning, but more important, trap placement should be made at the most favorable site to give maximum trap efficiency. Also needed as a guide is the knowledge of the probable maximum distance that male moths may be attracted to traps. A brief summary of these investigations will be presented here.

Experiments were generally carried out in areas free from the moth, both marked and unmarked males being released from a central point. On each of 12 lines radiating outward from the release point at 30° intervals was placed a trap, and the traps were moved away from the central point as they made catches. Males were sometimes recovered through woodland in traps ½ mile but much more often ⅓ mile away. Scouting traps should therefore be placed at intervals no greater than ½ mile, but preferably no greater than ⅓ mile.

As would be expected, the prime factor influencing moth flight appears to be wind direction. Traps situated upwind from an infestation are much more apt to recover moths than those down or across wind. Prevailing winds are generally from the south and southwest during the season of moth flight, and most captures were found in the 135° sector lying between south and northwest.

Terrain and local growth affect trap captures. It is advantageous to locate traps along hedgerows, wood edges, or at any point where there exists continuous wooded or bushy growth. In a typical experiment on such terrain moths were captured at about 70% of the traps, while only 25% of those located in open areas recovered moths. Males tend to avoid open country and to fly close to the ground for long distances along hedgerows or wood edges with occasional flights up and down tree trunks. Traps placed in hollows or surrounded by dense growth did not catch well, probably because of the restricted air movement.

EXPERIMENTAL TRAPPING.—Aside from the one variable under consideration it is important that the traps on lines set up to evaluate the performance of experimental traps be given an equal opportunity to capture moths.

Selection of Areas.—A prehatch survey of the intensity of infestation is made, and the trap lines are placed in those areas where sufficient moths will be present to supply adequate information. An even infestation is desirable, but the infestation must also be light so as to simulate the most critical conditions, those of incipient infestations. Furthermore, small differences in activity between treatments can be resolved more easily when

Table 1.—Males captured in traps baited with tips of females clipped at different times after emergence and stored under different conditions.[a]

HOURS AFTER EMERGENCE	1947 TESTS	1948 TESTS
0	42	61
In cellar (60° F. average temperature)		
8	110	150
16	293	27
24	112	139
32	264	281
40	285	214
48	239	164
56	263	228
64	340	252
72	364	399
In shed (70° F. average temperature)		
8	109	226
12	169	233
16	247	104

[a] Four replicates.

trapping is conducted in lightly infested areas. In some years when moths were expected to be too numerous, satisfactory results were obtained by applying a DDT-oil solution with a ground blower early in June so that infestations along roads to a depth of at least 200 feet were eliminated. Traps were subsequently set up in the cleared areas, and the number of moths caught during the season approximated that normally obtained in a lightly infested area.

Arrangement of Traps.—Traps were replicated four times. At first they were placed along roadsides at intervals of about 200 feet, but this procedure led to considerable variation in captures between replicates. Even a small line, when replicated four times, extended for such a long distance that competing traps were exposed to greatly varying moth populations.

In the method finally adopted, traps in each replicate were grouped together and each group was set up as a randomized block in separate lines, at least 400 feet apart. The individual traps in each line were placed 10 feet apart under as nearly the same conditions as possible. This procedure not only gave much better results, but it reduced the number of miles of roadside required to conduct the work and allowed far better placement of traps in the field. Only locations where infestation conditions were favorable were chosen.

Time of Moth Flight.—In tests conducted between 1947 and 1955 in Wendell State Forest in Massachusetts, the time of moth flight varied considerably. In 1953 traps set out on July 16 started to catch immediately and undoubtedly could have caught earlier; moths were taken until August 21 when the traps were removed. In 1948 traps did not start catching until August 5. In this area catches may be expected to start some time between mid-July and the first week in August. Traps are usually set out about July 20. Captures as late as September 25 were recorded in 1950.

Undoubtedly the time of flight depends greatly on the weather, especially on the temperature. The more northerly or cooler areas have later flights. However, the time of flight is not uniform even in the same general vicinity, flights in cooler areas being delayed. We found, like Collins & Potts (1932), that few males are trapped on rainy days or when the temperature is below 70° F. Usually the main flight takes place over a 10- to 14-day period; however, continued cool weather during the summer of 1956 stretched this flight period to about a month.

SEX LURE STUDIES.—The chemical makeup of the sex lure is not known. Investigations to determine its chemical identity have been reported by Acree (1953a, b, 1954) and are still in progress in our laboratories.

Collection of Sex Lure.—Heavy infestations of the gypsy moth are sought and female pupae are collected after they have aged sufficiently. The female pupae are readily distinguished from males by their much larger size, which is due to the eggs within their bodies. Single layers of pupae are placed on well-ventilated trays and periodically checked. The emerged moths are removed to a cage, and after aging for 16 to 24 hours at about 65° to 70° F., the tips are clipped into benzene, 1 ml. per tip being provided. An irritating fuzz is shed by the moth in the clipping operation; so face masks or other protective gear should be worn. Forced ventilation should also be provided to ensure removal of the toxic benzene vapors.

Effect of Age of Female Moths at Time of Clipping on Attractiveness of Tips.—Collins & Potts (1932) found that the time between emergence from pupation and clipping of tips affects the potency of the attractant. The data in table 1 confirmed their finding, that increased aging yielded a more attractive product.

Clipping of tips immediately upon moth emergence resulted in a lure of low potency. Although aging yielded generally more attractive materials, it also gave increased moth mortality. (Tips from dead moths are not attractive.) Thus, only 2% to 3% mortality was encountered after 16 to 24 hours and a 25% to 32% mortality after 64 to 72 hours. Currently moths are clipped between 16 and 24 hours after emergence.

Materials in the aging tests were not hydrogenated although lure used in the field is. It would probably be preferable to hydrogenate each of the extracts prior to testing. Extracts of tips, which caught practically no moths in the field, have become very active after chemical processing. If one infers that the attractant exists in free and bound forms with the latter being inactive, and that hydrogenation liberates the bound form of the lure, it is possible that after hydrogenation the lure from newly emerged moths may be as active as that collected from aged moths.

Bait Strength.—The effect of bait strength, expressed as number of tips per dispenser, on moth captures was tested over a period of 12 years. The bait strength required will, of course, depend on the efficiency of the trap used. The seasonal trap catches in some initial experiments are recorded blow:

Tips/Trap	*Average Catch (4 Replicates)*
2	16.8
4	46.3
8	53.0
16	72.5
32	77.3

These data indicate that there is no advantage in using more than 16 tips per trap. Therefore, the charge in

survey traps, originally set at 30 tips each, was reduced to 15 in 1947. Recently it was further reduced to 12 tips per trap, because tests made each summer over a 7-year period failed to demonstrate any significant difference between traps containing charges equivalent to 12 and 16 tips.

The following summary of seasonal moth catches in 1955, when the latest design of Graham trap was used, is typical:

Tips/Trap	*Average Catch (4 Replicates)*
8	25.5
12	42.3
16	44.3

With more than 30,000 traps being deployed each year in the gypsy moth eradication and control program, this reduction represents a considerable monetary saving.

Duration of Effectiveness of Lure.—A line of traps was set out to determine how early they could be placed in the field in advance of moth flight without affecting their efficiency, particularly during the latter part of the season. On June 20, a date well in advance of actual trap placement in the field, four replicate 16-tip traps were set out and a similarly baited trap was added to each replicate at 10-day intervals until July 30. Results of this experiment are recorded below:

Date Set Out	*Average Catch*
June 20	65.8
30	61.8
July 10	34.0
20	23.5
30	48.5

Traps set out on June 30 not only made the first capture on August 3 but also made captures on September 5, the last day moths were recovered in this line. It is therefore assumed that traps can be placed in the field several weeks before normal flight begins without impairing bait attractiveness throughout a normal season.

Lure from Foreign Sources.—The first collections of lure used in survey trapping were made in this country. However, it was recognized that the more the population of the moth was depressed by control measures the more difficult would the collection of pupae become. Consequently foreign sources of the attractant were explored during the early phases of this program. Collections have now been made in Spain, French Morocco, Portugal, and Yugoslavia, and the potency of these products has been found to be similar to that of domestic lure.

Efficiency of Lure from Laboratory-Reared Moths.—One of the possibilities still under consideration is the rearing of gypsy moths to provide the sex lure needed for the survey program. Lure prepared from laboratory-reared moths was found to be superior to that obtained in the field. In one study 15-tip charges prepared from each of three groups of moths raised in the laboratory during the winter and spring months were compared with currently collected field material, and gave the following results:

Approximate Date of Clipping	*Average Catch*
Feb. 15	51.3
Apr. 1	50.8
May 1	59.3
Current field	32.0

In these tests the lure was not hydrogenated.

Apparently forced hatching of egg masses and subsequent rearing of gypsy moths out of season under laboratory conditions does not adversely affect the potency of the sex attractant. However a satisfactory method for mass-rearing the gypsy moth has not yet been devised, mainly because of the high incidence of the polyhedral virus causing wilt disease, that is fatal to the larvae.

Activity of Whole-Moth Extracts.—Although extracts of the last two abdominal segments of the virgin female are effective, similar extracts of the entire moth are practically ineffective.

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The Status of San Jose Scale Parasitization[1] (Including Biological Notes)

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Abstract

The marked reduction in abundance of *Quadraspidiotus perniciosus* Comstock in North America during the years subsequent to its accidental introduction from China was correlated with its acquirement of a parasitic fauna. The most important members of this fauna are the ectoparasites, *Aphytis diaspidis* How. and *A. mytilaspidis* How., and the endoparasites, *Coccophagoides kuwanae* (Silv.) and *Prospaltella perniciosi* Tower. *P. perniciosi*, and its host, *Q. perniciosus*, presumably are native to China. Both were accidentally introduced into Australia, North America, and Brazil. *P. perniciosi* has been purposefully introduced into Chile, Europe, and South Africa. San Jose scale parasitization evidently caused a reduction in the incidence of infestations and enhanced the efficiency of control measures.

At the beginning of the present century a population explosion of the San Jose scale, *Quadraspidiotus perniciosus* Comstock, threatened the destruction of apple, pear, peach, and plum trees throughout the world (Marlatt 1906). This explosion began about 1873 with the importation into California of flowering peach trees bearing a few individuals of this insect. The peach trees came from the native habitat of the San Jose scale in northeast China where, as pointed out by Marlatt, its population was held at low densities by natural means. Thence the scale was carried, without its natural enemies, on nursery stock to most, if not all, the world's fruit-growing regions.

In California, and then in eastern United States, San Jose scale populations flared up in virulence and destruction and then subsided. Marlatt (1906) anticipated this phenomenon for each new area, and visualized the destructiveness of the San Jose scale being reduced in each by the action of natural enemies derived from allied coccids already present. He assumed, however, that its control by insecticides would always be necessary if salable crops were to be produced. Fortunately, this did not prove to be the case. As Hodgkiss & Parrott (1914) pointed out, the parasitization of the San Jose scale varies greatly with different localities, in kinds of parasitic species, in the numbers of each, and in their relative importance.

To the ecologist the worldwide explosion of the San Jose scale demonstrated the principle as stated by Brues (1908) that "The balance maintained by the struggle for existence between species is immediately and violently disturbed if the parasites of any particular species be removed. Such a form suddenly begins to increase in numbers, reproducing itself at a phenomenal rate approaching the geometrical progression, which would theoretically obtain if every individual were permitted to reach maturity and reproduce itself."

The Acquirement of a Parasitic Fauna.—The San Jose scale, after its establishment in North America, gradually accumulated a fauna of parasitic Hymenoptera totalling today some dozen species. Thus occurred one of the exceptions to Brues' (1908) generalization that the transfer of an insect from one region to another does not lay it open to attack from some of the forms closely related to parasites which control it in its native environment.[2] Such an exception, as Brues points out, derives from the fact that "throughout the entire parasitic group there are scattered here and there species which are particularly adaptive in that they attack several or a number of more or less closely related hosts."

The San Jose scale, soon after its establishment in North America, was attacked by two species of ectoparasites, *Aphytis diaspidis* How. (=*fuscipennis* How.) and *A. mytilaspidis* How. (Johnson 1900, Quayle 1911). Undoubtedly, in the early years of the San Jose scale explosion they played an important part in its reduction. In the fall of 1899 *A. diaspidis* had become so abundant in certain orchards in Maryland that growers generally were recommended to prune before spraying and save every twig bearing a scale in such a way that any parasites therefrom would fly back to the trees and attack the scale missed by the spray (Johnson 1900). The San Jose scale for about 60% of its life is suitable for the reproduction of ectoparasites such as *Aphytis*. This is in marked contrast to the California red scale, *Aonidiella aurantii* (Mask.), which is thus suitable for only about 10% of its life (Quayle 1910).

The parasitic species reared only occasionally from San Jose scale collected in North America are as follows: *Prospaltella aurantii* (How.) and *Aspidiotiphagus citrinus* (Craw), species which supposedly had been introduced into California from Japan during the 1880's in yellow scale, *Aonidiella citrina* (Coq.), infesting citrus nursery stock (Flanders 1956), *Coccophagoides similis* (Masi) and *Anabrolepis zetterstedtii* (Westw.), presumably originating in Europe, and the presumably native species, *Physcus varicornis* (How.), *Prospaltella berlesei* (How.) (here considered synonymous with *Prospaltella diaspidicola* Silv.), *Prospaltella murtfeldtae* (How.), and *Coccidencyrtus ensifer* (How.) (see Hodgkiss & Parrott 1914, McDaniel 1919, Rice 1937).

The most important known endoparasites of San Jose scale are the Chinese species *Coccophagoides kuwanae* (Silv.) and *Prospaltella perniciosi* Tower. The first occurrence in North America of *C. kuwanae* was reported by Rice (1937), that of *P. perniciosi* by Tower (1914).

The University of California Department of Biological Control in 1950 imported into California from Tsingtao, Shantung Province, China, San Jose scale parasitized by *Coccophagoides kuwanae* and a species of *Coccidencyrtus*. Scales from a native host plant, *Poincirus trifoliata*, yielded 14 females and four males of *Coccophagoides kuwanae* and two females of the *Coccidencyrtus*. Scales collected from peach trees in the same area yielded 11 females of *Coccophagoides kuwanae*. These females in California produced after mating a generation of female

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[2] Species so closely related that they can be distinguished with difficulty or not at all are likely to possess different species of parasites. This is the case with two biologically distinct forms of *Pseudococcus maritimus* Ehrh. occurring in California, a host-plant-specific form (characterized by diapause) infesting grape, and a nonhost-plant-specific form (without diapause) infesting apples, citrus, grape, pear, etc. (A. J. Basinger, unpublished notes). The encyrtid *Acerophagus notativentris* (Gir.), for example, is a highly effective parasite of the former but is unable to complete its development in the latter (Flanders 1944b).

progeny on insectary-grown San Jose scale. The male of *C. kuwanae* supposedly develops as an ectoparasite on the female of its own species, as R. L. Doutt (unpublished notes) has found to be the case with another species of the genus.

BIOLOGICAL FEATURES OF *PROSPALTELLA PERNICIOSI*.—*Prospaltella perniciosi* was first known to occur in China in 1949 when it was reared from the California red scale collected by the University of California Department of Biological Control in the vicinity of Canton, Kwangtung Province. It was then found that *P. perniciosi*, which universally is a uniparental species, consists of at least two host-determined races (Flanders 1950), one race being able to reproduce generation after generation on San Jose scale but not on California red scale, the other doing so on California red scale but not on San Jose scale. Occasionally, however, one of these races may reproduce in the second-instar males of the host of the other race.

Under natural conditions these host-determined races of *Prospaltella perniciosi* are differentiated by the bisexuality of the San Jose scale race and the unisexuality of the California red scale race. The males of *P. perniciosi* develop only as endoparasites of the female of their own species (Rice 1937).

Prospaltella perniciosi has a long host-searching period—up to 4 weeks at temperatures of about 80° F., it oviposits in all the nymphal instars and in the adult female of the host. Its reproductive capacity is about 50 progeny per parent without the parent having host-fed. The female can survive exposures to 24° F. and will actively search for hosts at temperatures less than 55° F.

At a constant temperature of 80° F. the life cycle of *Prospaltella perniciosi* is about 30 days if the egg is deposited in the first-instar host, 18 days if the egg is deposited immediately before the host scale is half grown, and 26 days if the egg is deposited after the host is half grown.

Prospaltella perniciosi apparently disperses readily. It was not long after its discovery in the North Atlantic states (Fernald 1914) that it was found throughout the San Jose scale-infested areas east of the Rocky Mountains. Somehow it was transported to South America where it parasitized San Jose scale infestations east of the Andes. Compere (Flanders 1944a) reported that in Brazil he was considerably impressed with its controlling effect on San Jose scale on plum trees.

The only region outside of the Americas where *Prospaltella perniciosi* occurs without having been purposefully introduced is Australia. There, under the name of *Coccophagus clariscutellum* (Gir.), it is reported as an abundant parasite of San Jose scale in Queensland (Jarvis 1927). Subsequently in 1931 it was reared from red scale material collected by the writer from orange trees in New South Wales.

During the past two decades *Prospaltella perniciosi* has been purposefully introduced into at least five additional regions: South Africa 1941–42 (Annand 1942), California in 1943 (Flanders 1944a), Italy in 1946 (exportation records Univ. Calif. Dept. of Biol. Control), Germany in 1950 (Wolfgang Klett, *in litt.*), and Chile in 1959 (Roberto Bobadillo Toro, *in litt.*). In 1955 the writer reared *P. perniciosi* (tentative identification) from *Chrysomphalus dictyospermi* (Morg.) infesting several leaves of an olive tree in the garden of the Villa Jaccarino in Sorrento, Italy. Twenty per cent of these scales had been parasitized.

BENEFICIAL ROLE OF PARASITES.—Experimental data evaluating the biological control of San Jose scale are lacking. As late as 1950 Harry S. Smith, as Chairman of the Department of Biological Control of the University of California, wrote to Wolfgang Klett of Germany as follows: "While it is true that the San Jose scale is a very minor pest in California whereas formerly it was a major pest, no careful study has been made to prove that this is due to parasites. I must say, however, that I can see no other explanation."

Parasites of San Jose scale may occur in northeast China which are more effective than those now present in North America. Pending the introduction of such parasites, the fruit grower should give full credit to those presently feeding on the San Jose scale on his fruit trees. As pointed out by Compere (1955), biological control workers in earlier years appraised the controlling effect of natural enemies as either effective or noneffective, as if there were no intermediate degrees of control. In reality there are probably many degrees of effectiveness. It seems evident that some pests would be far more injurious than they are at present were it not for the so-called ineffective parasites that prey upon them.

It is evident historically that when the San Jose scale invaded certain new habitats it acquired a parasitic fauna which caused so great a reduction in its population pressure that the incidence of infestation was diminished and the efficiency of control measures enhanced.

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A Wheat Germ Medium for Rearing the Pink Bollworm[1]

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ABSTRACT

Composition of an artificial medium for laboratory cultures of the pink bollworm, *Pectinophora gossypiella* (Saund.), is given. The medium has wheat germ meal as the primary constituent but also contains vitamin-free casein, sucrose, Wesson's salts, choline chloride, agar, sodium alginate, vitamins, and water. Growth and development on the pink bollworm on this diet approximated that reported for the insect in the field. The larval developmental period averaged 16.3 days; prepupae 2.2 days; and pupae 8.8 days. Pupae produced on this medium were slightly smaller than those collected from cotton bolls. Oviposition records indicated that females reared on the wheat germ medium would produce more eggs than field-collected moths. Records for several thousand insects indicated that an average of 81.5% of the larvae originally placed on the diet would grow into adults.

There has been considerable research directed toward the development of chemically defined artificial media suitable for laboratory-rearing of the pink bollworm, *Pectinophora gossypiella* (Saund.). Beckman *et al.* (1953) reported that the pink bollworm could be reared on a medium composed mainly of a modified chick ration having egg albumin as the protein constituent. However, development of the insect was slow and the number of adults produced was small with regard to the number of eggs originally placed on the medium. Vanderzant & Reiser (1956a) developed a rearing medium based on albumin on which apparently normal pink bollworms could be produced. The developmental period and pupal weights approximated those reported for pink bollworms in the field. The results of this important research developed many of the principles on which later work was based. Vanderzant *et al.* (1956) reported a method for mass-rearing of the pink bollworm on sprouted peas under aseptic conditions. However, this technique had certain disadvantages since the preparation of the seed was time consuming and great care had to be exercised to avoid contamination. Research by Beck & Stauffer (1950) which led to a purified casein medium for the European corn borer, *Pyrausta nubilalis* (Hbn.) provided the basis for the development by Vanderzant & Reiser (1956b) of a similar type purified casein medium on which the pink bollworm could be successfully reared. The casein medium not only provided a method for future work pertaining to the dietary requirements of the pink bollworm, but it also proved valuable in the development of a rearing medium for laboratory cultures of the boll weevil, *Anthonomus grandis* Boh. (Vanderzant & Davich 1958). The casein diet also provided the basal medium for a study of the role of dietary fatty acids in the development of the pink bollworm (Vanderzant *et al.* 1957). It was concluded from this work that the inclusion of corn oil in the medium would meet the insect's requirement for the essential fatty acid, linoleic acid. In subsequent experiments, the amino acid requirements of the pink bollworm were determined (Vanderzant 1957, 1958).

Table 1.—Composition of two wheat germ diets compared with the casein diet for rearing the pink bollworm.

CONSTITUENTS	GRAMS		
	Casein	Wheat Germ 1	Wheat Germ 2
Casein, vitamin free	5.00	3.0	3.5
Cysteine hydrochloride	0.10		
Glycine	0.15		
Wheat germ		3.0	3.0
Sucrose	5.00	5.0	3.5
Salts, Wesson's	1.20	1.0	1.0
Cholesterol	0.05		
Corn Oil	0.25		
α-Tocopherol	0.01		
Choline chloride	0.10	0.1	0.1
Cellulose	4.00		
Agar	3.00	2.0	2.5
Sodium alginate	0.50		0.5
Vitamin mixture[a]	1.0 ml.	1.0 ml.	1.0 ml.
Water	80.0 ml.	80.0 ml.	85.0 ml.

[a] The vitamin mixture used for the casein and wheat germ 1 media did not contain inositol.

Results of the investigations reviewed above led to the development of the wheat germ medium. This latter medium contains fewer ingredients and requires less rigid rearing conditions than the casein medium. Synthesis and preliminary testing of the medium under aseptic conditions was performed by Vanderzant.

COMPOSITION OF MEDIUM.—The composition of the wheat germ medium is relatively simple when compared with the casein diet developed earlier by Vanderzant & Reiser (1956b). This is well illustrated by the list of constituents cited for each medium in table 1. The major constituent is wheat germ which contains sterols, essential fatty acids, and tocopherols. In addition, it also supplies protein, carbohydrates, vitamins, and minerals.

During preliminary testing, two wheat germ media were studied. Cold-rolled wheat germ was used in all tests except one in which heat-treated wheat germ (commonly

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Table 2.—A comparison of pupal weights of pink bollworm reared on wheat germ and casein media with pupa collected from cotton bolls.

Source	Average Weight in Milligrams				
	No. of Females	Pupal Weight	No. of Males	Pupal Weight	Average Weight
Wheat germ 1[a]	337	23.0	191	18.0	20.5
Wheat germ 2[a]	124	22.4	60	18.5	20.5
Wheat germ 2[b]	75	21.7	75	16.1	19.5
Casein[a]	110	21.3	85	16.2	18.8
Field[c]	50	—	—	—	23.9

[a] Insects reared aseptically.
[b] Insects reared nonaseptically on diet containing the inhibitor solution.
[c] Unsexed.

sold as cereal) was tested. Although the latter had a lower lipide content, results were similar to those obtained when the cold-rolled wheat germ was used. The differences in the two wheat germ media are indicated in table 1. With the exception of the pupal weights presented in table 2, all records were taken of insects reared on wheat germ 2 medium.

The vitamin mixture was similar to that developed in earlier work (Vanderzant 1957) except the mixture used for the wheat germ medium included inositol since the latter is needed by the boll weevil which is also reared in this laboratory (Vanderzant 1959). Composition of the vitamin solution is presented in table 3.

During preliminary testing, all the insects were reared under aseptic conditions. In subsequent tests, a solution containing chemicals which inhibited the growth of contaminating microorganisms was added to the media. The inhibitor solution contained sorbic acid, methyl paraben (methyl parahydroxybenzoate) and butyl paraben (butyl parahydroxybenzoate) dissolved in 95% ethyl alcohol (Clark 1958). The inhibitors were added to the diet at a concentration equal to 0.2% by weight of each. The inhibitor solution was usually made up in quantities of 100 ml. or more. The proper concentration of the solution was pipetted in each batch of medium as it was being mixed. The use of the inhibitors plus clean equipment and careful technique allowed large numbers of insects to be reared nonaseptically with little contamination.

Preparation of the Media.—Dry ingredients for the medium were weighed separately on a torsion balance. Agar was dissolved in the required amount of cold water and heated to the boiling point. The remainder of the dry ingredients were placed in a Waring Blendor and the boiling water-agar mixture was then added. Other liquid ingredients were pipetted into the mixture and allowed to blend for several minutes. After blending, the hot mixture was poured into plastic "squeeze" bottles. This type bottle was used for dispensing media to the rearing vials. The spout of the plastic bottle was inserted into clean 2-dram shell vials and the media dispensed into them. The vials were filled to about one-third of their volume with medium.

Table 3.—Composition of the vitmain mixture incorporated in the wheat germ media.

Constituent	Mg. per 100 Grams of Medium
Niacinamide	1.00
Calcium pantothenate	1.00
Thiamine (HCl)	0.25
Riboflavin	0.50
Pyridoxine (HCl)	0.25
Folic acid	0.25
Biotin	0.02
Vitamin B_{12}	0.002
Inositol	20.00

The techniques outlined by Vanderzant & Reiser (1956a, 1956b) were used for rearing larvae aseptically. The vials containing media were plugged with nonabsorbent cotton and autoclaved. After the vials had cooled, one newly hatched larva was placed in each. The larvae were incubated from eggs which had been collected and treated with a sterilizing solution according to the technique of Vanderzant & Reiser (1956a). The vials were kept in an incubator in which a temperature of approximately 85° F. and a relative humidity of 75% to 85% was maintained. The incubator maintained a light cycle of 12 hours of light followed by 12 hours of dark.

Techniques for rearing larvae on the media containing the inhibitor solution were somewhat different from that outlined above. The vials were either heat sterilized or autoclaved before the medium was added to them. After the hot medium was added, the vials were covered with paper and left at room temperature until all free moisture on the sides of the vials had evaporated. Larvae were obtained from eggs washed only in distilled water. The larvae were placed singly in the vial by means of a soft camel's-hair brush. During this process the brush was dipped into an antiseptic solution containing 3000 p. p. m. "Roccal" (benzalkonium chloride) at frequent intervals. After the larvae were transferred, each vial was plugged with clean nonabsorbent cotton and held under continuous light in an incubator at approximately 85° F. and 80% to 90% relative humidity.

Duration of the Developmental Period.—Daily observations of a number of pink bollworms reared on the wheat germ diet containing inhibitors were made to determine the average duration of each developmental stage. These data are reported in table 4. Each vial was numbered so that records could be maintained for each larva and pupa during the entire developmental period. Final records include only insects that pupated within a 60-day test period. Individuals in vials that became contaminated with microorganisms were discarded and were not included in the records. The insects were reared under the conditions previously described for nonaseptic rearing.

Records indicated that larval development required an average of 16.3 days for completion with a range of 13 to 26 days. The mode indicates that the greatest number of individuals completed larval development in 18 days. Only four insects out of the 75 observed required more than 19 days to complete larval development. These data indicate the rapid and uniform rate of development of pink bollworm larvae that may be expected when the insect is reared on wheat germ medium. The duration of the active feeding period on the wheat germ medium is comparable to that reported on bolls and shorter than that reported on squares (Fenton & Owen 1953).

The prepupal stage required an average of 2.2 days and the pupal stage 8.8 days. These figures are about the same

Table 4.—Duration of the immature life history stages of the pink bollworm when reared on a wheat germ diet as compared with those reared on cotton bolls and squares.[a]

Stage	Number of Insects	Diet	Days		
			Average	Mode	Range
Larval	75	Wheat germ[b]	16.3	18	13–26
Prepupal	75	Wheat germ[b]	2.2	2	1– 5
Pupal	75	Wheat germ[b]	8.8	8	5–11
Larval	958	Squares	10.1		6–18
Prepupal	958	Squares	3.8		1–26
Pupal	958	Squares	8.1		6–24
Larval	878	Bolls	16.5		11–25
Prepupal	878	Bolls	3.3		1–26
Pupal	878	Bolls	8.6		6–24

[a] Life history records from bolls and squares were taken from data reported by Fenton & Owen (1953).
[b] Insects reared nonaseptically on diets containing the inhibitor solution.

as those reported for boll- and square-fed pink bollworms by the above authors.

PUPAL WEIGHTS.—Pupal weights for pink bollworms reared aseptically and nonaseptically on wheat germ media are compared with those obtained from cotton bolls grown in the field. Pupae were weighed in milligrams on a Roller-Smith balance. Weights of the pupae are given in table 2. The average weights of pink bollworm pupae reared aseptically on wheat germ media 1 and 2 indicated little difference between the media. The wheat germ medium produced pupae slightly larger than the casein medium and slightly smaller than recorded for pupae recovered from cotton bolls.

Pupal weights obtained from a large number of insects reared for four consecutive generations indicate no deleterious effects from continuous rearing on wheat germ media (table 5).

OVIPOSITION RECORDS.—One pair of moths from the wheat germ medium was confined without food or water on cotton terminals in small glass enclosed cages. Fresh terminals were placed in the cage every 3 days. The terminals, after removal from the cages, were closely examined under a binocular microscope and the number of eggs counted. Moths produced from larvae collected from field-grown cotton bolls were used for comparison. Oviposition records are reported in table 6. Females produced on the wheat germ media had a higher average rate of oviposition than the moths collected from the field, the average number being 62.4 eggs and 32.2 eggs per female, respectively. Published data indicated that considerably greater numbers of eggs could be expected if the moths were furnished water or a sugar solution (Fenton & Owen 1953, Lukefahr & Griffin 1956).

Table 5.—Average pupal weights of four continuous generations of pink bollworms reared on a wheat germ medium containing inhibitor solution.

Generation	Pupal Weights in Milligrams				
	No. of Males	Pupal Weight	No. of Females	Pupal Weight	Population Average
1	238	17.8	479	19.8	19.1
2	243	20.1	596	21.2	20.9
3	237	20.7	430	21.7	21.3
4	150	20.4	418	21.9	21.5

Table 6.—Oviposition records of female pink bollworms reared on a wheat germ medium compared with moths collected from the field.

Source	No. of Females	Eggs per Female	Range
Wheat germ	48	62.4	9–168
Field	19	32.2	2–91

Table 7.—Yields of mature pink bollworms as a percentage of the total number of larvae originally placed on a wheat germ medium containing inhibitor solution.

Batch Number	No. of Vials Infested	No. of Insects Recovered	Per Cent Yield
1	1,161	877	75.5
2	1,292	1,096	84.8
3	993	845	85.1
4	432	344	79.6
Total	3,878	3,162	81.5

YIELD AND CONTAMINATION.—An important consideration in using any artificial diet is the number of mature insects produced in proportion to the number of eggs or larvae originally placed on it. The data presented in table 7 for insects produced on four different batches of wheat germ media indicate that approximately 75% to 85% of the larvae can be expected to live and become adults on any given batch of medium. The overall average yield of adults for the media was 81.5%. Larval death on the media was attributed to several factors, *viz.*, inherent weakness in certain larvae, time elapsed between incubation and transfer to media, larval entanglement in the cotton plugs, larval injury during transfer from incubation chamber to media, larval escape from rearing container, contamination due to poor technique, and mite infestation in certain vials which produced contamination. From experience with other media, the yield of adults reared on the wheat germ medium is considered excellent.

Contamination of the rearing medium by microorganism will lower the yield and impair its usefulness as a laboratory tool. Contamination was usually traceable to poor technique or mite infestations. Good sanitation practices were sufficient to hold contamination at low levels when the antimicrobial agents were included in the diet.

REARING OF OTHER INSECTS.—Some measure of success in rearing certain other lepidopterous insects on the wheat germ media has been attained. No attempt has been made to modify the diet in order to make it a more efficient rearing medium for these species. Preliminary results indicate that the diet has possibilities for the laboratory production of a number of insects other than the pink bollworm.

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Effects of Low Doses of Ethylene Dibromide on Some Stages of the Confused Flour Beetle, *Tribolium confusum*[1]

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ABSTRACT

Eggs, pupae, and adults of the confused flour beetle, *Tribolium confusum* Duv., were exposed to low doses of ethylene dibromide in modified Strand flasks. Female adults were more susceptible than males to low doses of this fumigant and survivors laid few eggs during a postfumigation period of 10 days. Eggs laid by female survivors combined with male survivors showed reduced hatchability. Progeny from survivors developed normally. Hatchability of treated 1-day-old eggs decreased with increasing concentration but larvae from hatchable eggs developed normally. At the doses used, 4-day-old eggs failed to hatch. Eight-day-old pupae were more susceptible than 2-day-old ones and females more than males. Mature pupae showed abnormal darkening of the cuticle and abnormal development at metamorphosis. No strong evidence of a strain of flour resistance was found. The question of resistance in the field is discussed.

The primary objective of insecticide research is the effective control of insects. Because of this preoccupation with lethal effectiveness few people recognize that insects surviving insecticidal treatment may suffer physiological effects other than death. Therefore it is important to investigate the physiology of insects that have apparently recovered from the effects of insecticides. These survivors may suffer latent effects reflected in an impaired ability to develop or to lay eggs. On the other hand, insecticides with selective action may give rise to survivors whose progeny can tolerate doses that are lethal to a normal population.

Chemical control of insects in flour mills and grain elevators is achieved by fumigation more than by any other method. In flour mill "spot" fumigations the fumigant is poured into individual machines or sites known to be infested with all life stages of an insect. Under these conditions some insects survive, either because one life stage is more tolerant than another to a given dose or because they may escape the area of lethal concentration. If fumigation impairs the rate of development or the fecundity and fertility of survivors, control is better than that indicated by mortality data alone but if the regular and repeated use of a fumigant gives rise to a resistant strain changes in control procedures may become necessary.

Herrick & Horsfall (1931) showed that oviposition and egg hatchability of the bean weevil (*Acanthoscelides obtectus* (Say)) were stimulated by half-hour exposures to ethylene oxide. With longer exposures oviposition and hatchability decreased and the number of days that elapsed before viable eggs were laid increased. Horsfall (1934) described the effects of ethylene oxide on various stages of *T. confusum* and showed that more abnormalities were obtained from prepupae than from pupae. Treatment of adults caused a decrease in oviposition. Mackie & Carter (1937) demonstrated a "protective stupefaction" whereby a low concentration of gas caused a suspension of normal respiration in insects, thus protecting them against further action of the fumigant. Lindgren (1938) showed that this phenomenon occurred with the granary weevil, *Sitophilus granarius* (L.) but not with *T. confusum*.

The aims of this investigstion were to measure the rates of mortality and development of eggs, pupae, and adults of *T. confusum* exposed to low doses of ethylene dibromide, the number and hatchability of eggs laid by treated adults, and the rates of mortality, development, and oviposition of progeny from treated parents.

MATERIALS AND METHODS.—Strand (1930) developed a simple fumigation chamber consisting of a 6.4 liter Erlenmyer flask closed with a paraffined rubber stopper. Lindgren & Shepard (1932) improved it somewhat by using a ground glass fitting. A modified version of this flask shown in figure 1 proved satisfactory. The flask is fitted with a ground glass stopper to which are sealed two

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glass tubes extending 50 mm. above the stopper where they bend at right angles and extend 125 mm. in opposite directions. These tubes are fitted with stopcocks to regulate air pressure or to introduce and expel gas. The volume of each flask was calculated by water displacement.

Test insects of uniform age and condition were enclosed in plastic cylinders 30 mm. wide and 35 mm. in diameter sealed at the open ends with 60-mesh bolting silk. Insects were introduced through a hole drilled into the wall of the cage. A piece of doweling inserted into this hole and fastened to the long glass tube extending into the flask served to hold the cage in a fixed position.

A 1/4-dram shell vial containing a weighed amount of ethylene dibromide was stoppered with a cork wrapped in an impermeable membrane (Saran wrap) to prevent vapor loss. The vial was slid into the open flask which was then stoppered, slightly evacuated (755 mm. of Hg.) and shaken manually to break the vial against the inside wall of the flask. Under the slightly reduced pressure the released fumigant volatilized rapidly; it was considered that full concentration and uniform distribution were reached very quickly. Preliminary bioassay tests showed no significant differences in mortality of insects exposed at 3 levels in undisturbed flasks.

With this apparatus and technique it was considered that no gas was lost and that uniform concentration was maintained during almost the entire fumigation period. The median lethal dose of ethylene dibromide was calculated from probit-mortality curves to serve as a guide in determining low doses. Preliminary tests showed that 100% mortality occurred in 3 days at 5 mg. per liter and in 10 days at 3 mg. per liter of ethylene dibromide. Therefore all tests were conducted with doses of less than 3 mg. per liter.

Test insects reared at 27° C. and 70% relative humidity were held without food for 24 hours before fumigation to ensure uniform metabolic activity and thus a more uniform response to the fumigant (Tattersfield 1939, Crauford-Benson 1938). Adults were between 1 and 3 months old and those used in any one test were of comparable age. Because Sun (1947) found that temperature affects insect susceptibility to fumigants all fumigation tests were conducted at 27° C. After an exposure of 5 hours insects were transferred from the cages to Petri dishes containing culture medium and maintained at 27° C. and 70% relative humidity. To show the effect on fecundity and fertility of treating both sexes, treated and untreated males and females were combined in all possible ways. Daily observations during a postfumigation period of at least 8 days were made to determine mortality, fecundity, and fertility. Rates of development of eggs laid by treated and untreated females were compared. The mortalities and rates of development of treated eggs and pupae were determined.

RESULTS.—*Adult.*—Figure 2 shows the mortality of 100 virgin females and 100 males, 11 weeks old, and the mean number and hatchability of eggs laid by mated survivors in an 8-day period after exposure at 2 mg. per liter. Total mortality was significantly higher in females than in males ($\chi^2=34.9$, $\chi^2_{.01}=6.6$). On the first day after treatment male and female mortality was 0% and 3%, respectively, by the third day 3% and 6%, by the sixth, 36% and 44%, and by the eighth 44% and 85%. Treated

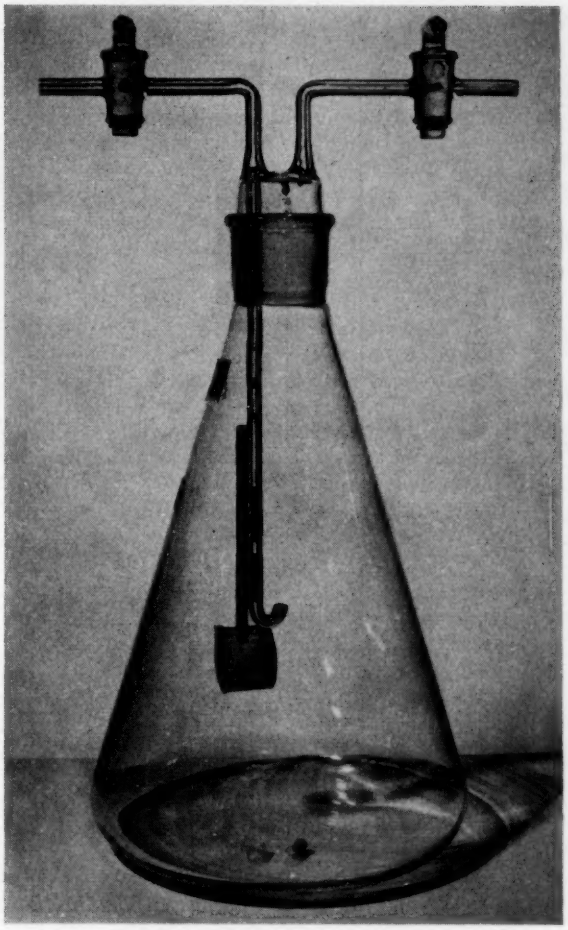

FIG. 1.—The fumigation assembly.

females laid significantly fewer eggs than untreated ones during this period ($t=3.8$, $t_{.01}=3.7$). Between the second and eighth day the mean numbers of eggs per day laid by treated and untreated females were 0.4 and 6.7, respectively. The hatchability of eggs laid by females beyond the second day after treatment was greatly reduced, the mean percentage values for eggs from treated and untreated females being 42% and 84%, respectively.

The cumulative per cent mortality during a postfumigation period of 8 days, of males and females, 6 1/2 weeks old, mated before and after exposure at 2 mg. per liter is tabulated below:

Day	1	2	3	4	5	6	7	8
Female	0	0	4	20	44	60	72	82
Male	0	0	0	2	10	28	34	46

There was a delayed lethal effect on both sexes, mortality beginning on the third day for females and on the fourth for males. Females died at a faster rate and in significantly larger numbers than did males ($\chi^2=12.8$, $\chi^2_{.01}=6.6$).

Table 1 shows the rate of oviposition of mated, treated females combined with treated and untreated males after exposure at 1.0, 1.5, and 2.0 mg. per liter. At all doses the mean rate of oviposition of treated females combined with treated or untreated males was significantly lower than that of untreated females combined with treated or un-

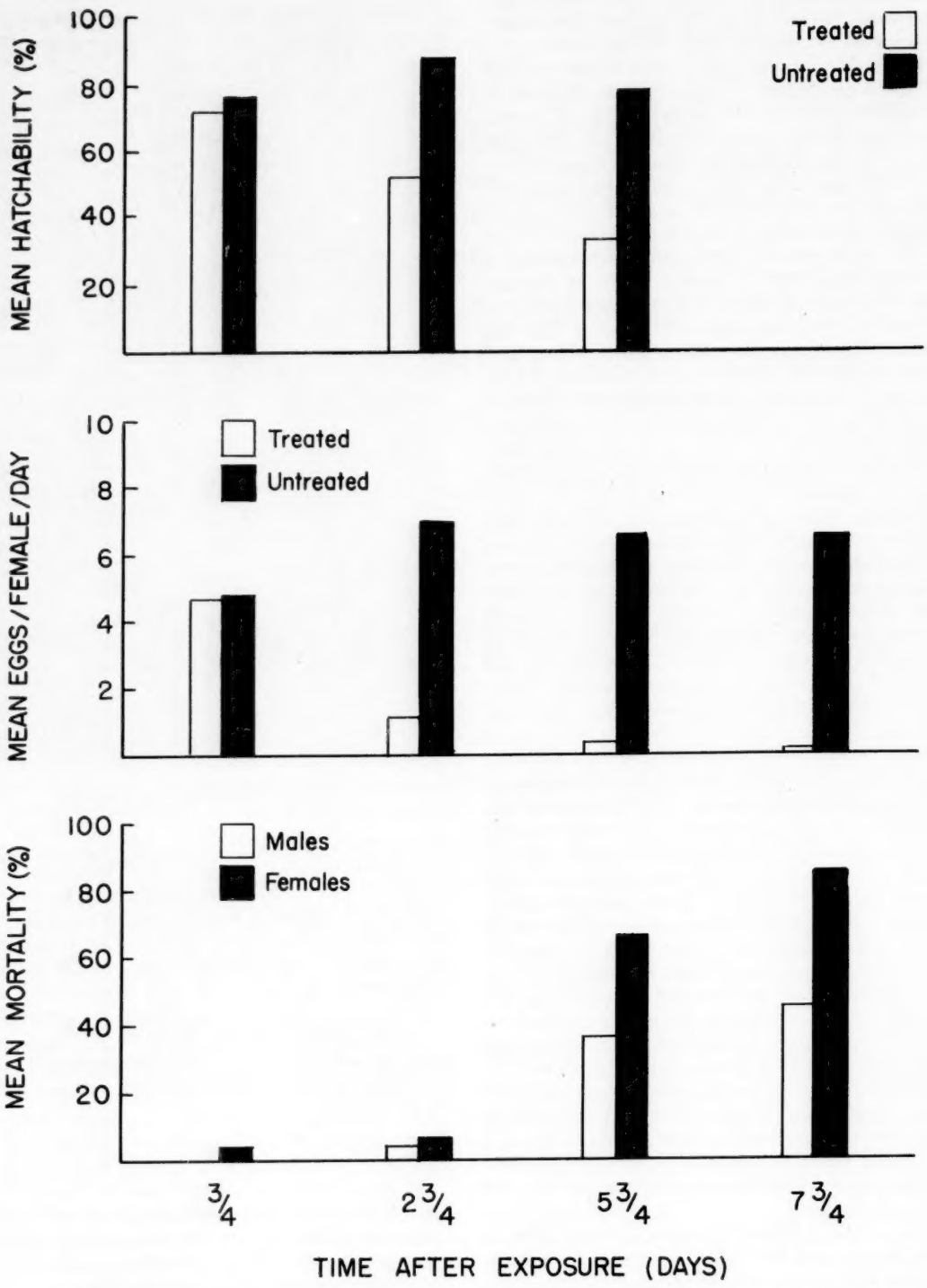

FIG. 2.—Mean mortality, fecundity and fertility of adults of *Tribolium confusum*, 11 weeks old, mated after exposure to ethylene dibromide at 2 mg. per liter.

Table 1.—Rate of oviposition of 25 mated, treated (T) females combined with 25 treated or untreated (U) males after exposure to three different doses of ethylene dibromide.

Dose (mg./l.)	Number of Eggs per Female per Day, Based on 10 Days				F-Values		
	T♂, T♀	U♂, T♀	T♂, U♀	U♂, U♀	T♀	T♂	Interaction of ♂ and ♀
1.0	4.8	5.7	8.9	8.9	102.0**	<1	1.5
1.5	1.3	1.5	7.5	7.1	252.0**	<1	<1
2.0	0.9	0.8	5.6	5.5	601.0**	<1	<1

** Significant at 1% level.

treated males. These data including the highly significant F-values for females show that reduced oviposition was due entirely to the effect of treatment on females, treatment of males having no significant effect in reducing the fecundity of treated or untreated females. At 2 mg. per liter the daily data on fecundity showed that the rate of oviposition was reduced within 3 days after treatment and continued lower than that of untreated females for the remainder of the postfumigation period of observation. Oviposition was reduced before any appreciable mortality had occurred.

Data in table 2 show that normal hatchability occurred in eggs laid by insects surviving exposure to 1.0 mg. per liter but there was a significant reduction in the hatchability of eggs from parents surviving doses of 1.5 and 2.0 mg. per liter. Treatment of males had no apparent effect on egg hatchability. It is interesting to note that egg hatchability was reduced only if both parents were treated.

Eggs laid by mated females exposed at 1.0 or 1.5 mg. per liter gave rise to larvae that developed normally and only slightly slower than larvae from eggs laid by untreated females. The developmental period from egg to adult of eggs from parents exposed at 1.5 mg. per liter and from untreated parents was 42.5 and 41.7 days, respectively.

Egg.—Ethylene dibromide at 0.5 mg. per liter had no effect on the hatchability and rate of development of 250 treated 1-day-old eggs. Pupal and adult emergence were normal. At 1.0 mg. per liter hatchability of 1-day-old eggs was only 44% but the larvae developed normally. At both doses 4-day-old eggs failed to hatch.

The number and hatchability of eggs laid by adults that developed from eggs exposed at 0.5 mg. per liter were normal.

Pupa.—Pupae 0 to 2 days old exposed at four concentrations ranging from 0.5 to 2.0 mg. per liter had a mean adult emergence of 97%. The rate of development of male and female pupae was unaffected by any dose within this range. At 1.0 mg. per liter adult emergence of prepupae was 85% but at 1.5 and 2.0 mg. per liter it was 70%. Adults that emerged from 1-day-old pupae exposed at these concentrations laid normal numbers of eggs after the first week of emergence.

Eight-day-old pupae were more susceptible than 2-day-old ones exposed at 2 mg. per liter and females more than males. Six days after treatment male and female pupal mortalities were 34% and 42%, respectively, male and female adult emergence 50% and 26%, respectively. Many pupae showed abnormal development (Fig. 3). These were considerably darker than normal pupae of the same age. Hardening and darkening of the cuticle appeared more advanced in the head and thorax than in the abdomen. At metamorphosis adult characters were well developed in the head and thorax but not in the abdomen. Most of them died while attempting to extricate themselves from the pupal skin and none lived more than 1 or 2 days after adult emergence.

Table 2.—Hatchability of eggs laid by 25 mated treated (T) females combined with 25 treated or untreated (U) males after exposure to three different doses of ethylene dibromide.

Dose (mg./l.)	Per Cent Hatchability (Based on 10 Days)			
	T♂, T♀	U♂, T♀	T♂, U♀	U♂, U♀
1.0	84	84	84	83
1.5	60[a]	84	81[a]	83
2.0	61[b]	72[b]	78	77

[a] $\chi^2 = 53.0$, $\chi^2_{0.01} = 6.6$.
[b] $\chi^2 = 4.5$, $\chi^2_{0.05} = 3.8$.

DISCUSSION.—The number and hatchability of eggs laid by treated females on the first day after exposure to ethylene dibromide were normal. This may mean that at the time of treatment the eggs were mature and at this stage of development were unaffected by treatment, or that the effects of low doses are not immediately apparent.

The greater susceptibility of females than males and the reduced fecundity and fertility of survivors are important from the practical standpoint. The ability of ethylene dibromide to produce these results makes it a useful fumigant to retard the rate of population increase in flour mills.

Gough (1939) noted that females of *T. confusum* are more susceptible than males to hydrogen cyanide. Park (1936) found that females of *T. confusum* have a higher respiratory rate than males. This may account for the higher susceptibility to ethylene dibromide of the female. However, this difference in the susceptibility of the sexes was found also with *p-p'*DDT (Loschiavo 1955). Horsfall (1934) found a decrease in oviposition of *T. confusum* exposed to ethylene oxide and concluded that inhibited oviposition resulted from the effects produced on females rather than on males.

The mode of action of ethylene dibromide in reducing oviposition in *T. confusum* is unknown but, since this fumigant is an excellent fat solvent it may alter the lipid material chemically thus rendering it unavailable for egg maturation.

Reduced hatchability of eggs laid by treated females was at first attributed to reduced copulations among survivors. However the females had been mated for 6 days before treatment and should have had a sufficient store of sperms (Sinha 1953) to fertilize eggs after treatment even if no further copulations took place. As table 2 shows, hatchability was reduced only if both parents were treated. If treatment of females is responsible for reduced egg hatchability then eggs laid by treated females combined with untreated males should show reduced hatchability also. This was not shown in table 2 although a trend towards lowered hatchability was indicated at the 2.0 mg. per liter dose. Perhaps at higher doses the effect would be more pronounced.

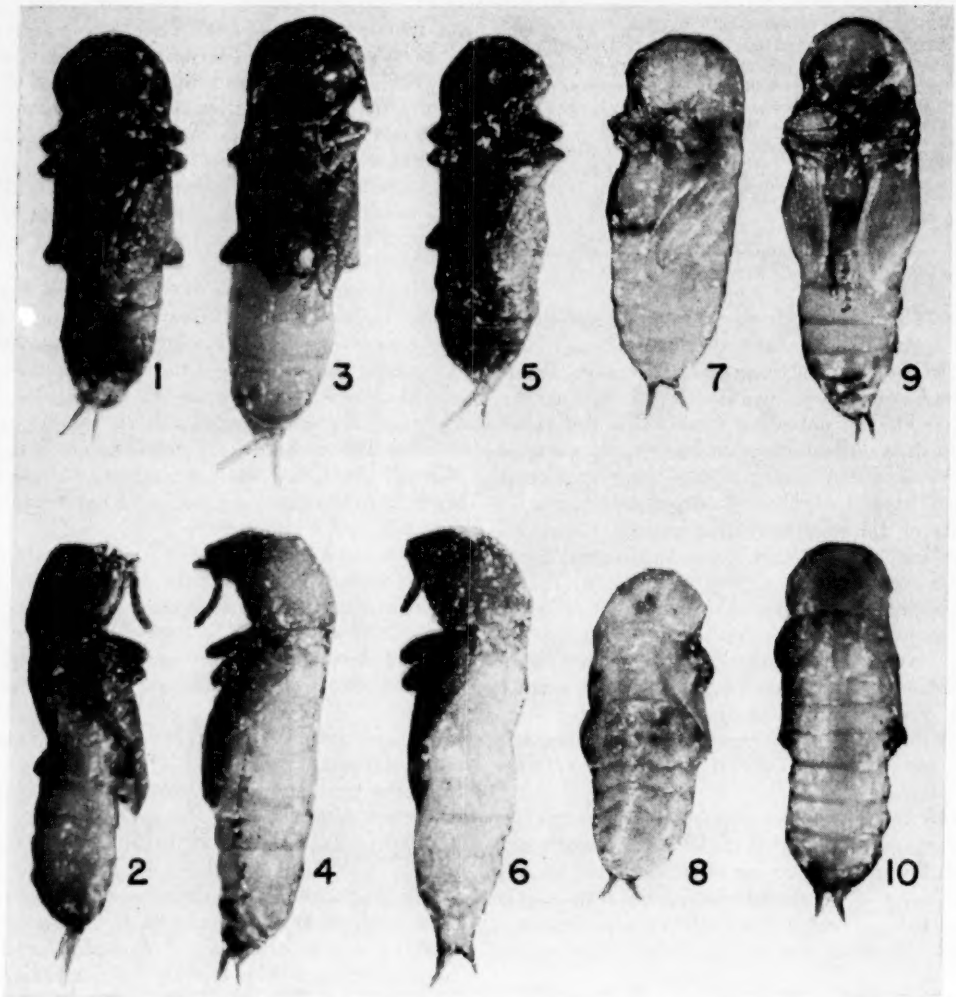

FIG. 3.—Pupae after exposure to ethylene dibromide at 2 mg. per liter; top row ventral view, bottom row dorsal view; 1–6 treated, 7–8 untreated 1-day-old pupae, 9–10 untreated 8-day-old pupae.

The failure of 4-day-old eggs to hatch after exposure to doses of 0.5 and 1.0 mg. per liter is probably related to increased rates of metabolism and respiration. Sun (1947) found that older eggs of *T. confusum* were more susceptible than younger ones to carbon disulphide. He states that several workers have shown for various insect eggs that there was an early "formative" period characterized by a low respiratory rate and a later period, in which the metabolism increased up to the time of hatching.

The greater susceptibility of 8-day-old than 2-day-old pupae may be correlated with a higher oxygen uptake of the older pupae. Lindgren (1935) showed that the rate of respiration of *T. confusum* pupae is high at first, then falls for several days and then rises again near the end of the pupal period.

The phenomenon of abnormal darkening of the integument of treated pupae may involve an interaction between ethylene dibromide and the polyphenol oxidases concerned with darkening and hardening of the cuticle. The relatively slower development and slower darkening of the abdominal integument indicate that the hormone system concerned with molting and metamorphosis (Wigglesworth 1934) are affected in some way. Gough (1939) found that pupae of *T. confusum* which had been fumigated with hydrocyanic acid frequently gave rise to adults with undeveloped elytra.

Resistance.—Because the milling industry generally is carrying out a regular and effective program of spot fumigation it is difficult to obtain enough survivors to conduct selection experiments. The few observations that have been made have not indicated the presence of a strain of flour beetles resistant to ethylene dibromide. Fumigants give steep dosage-mortality regression curves showing that the range of doses which will exert selection pressure on the population is very narrow. This would indicate the absence of genotypic variation in response to ethylene dibromide. Cotton (1958) states that no evidence of the development of resistance of stored products insects has been observed. However Monro & Upitis (1956) selected a strain of granary weevil more resistant to methyl bromide than the stock from which it was derived although the $LD_{99.9}$ was only four times higher after 23 generations of selection. This may be an example of "vigor tolerance" rather than of genotypic resistance. Reports from indus-

try that repeated applications of fumigants no longer give satisfactory control indicate that selection may occur in the field. Gough found experimentally that the offspring of individuals resistant to hydrogen cyanide were significantly more resistant to this fumigant than the offspring of susceptible individuals, and that this difference was maintained over several generations. Certainly more laboratory and field work are necessary to determine whether resistsnce to fumigants exists among stored-product insects.

If resistant genotypes do occur in flour beetles the absence of a buildup of resistance in the field may be due to the heterogeneity of a field population due to the mechanics of mill operation. In practice, insects, including survivors of fumigations, remain with the coarser milling products and eventually accumulate at the tail end of the mill. They are thus prevented from infesting the higher mill stocks where they may breed a selected strain. Also, there may be a residual population in the mill not reached by the fumigant or new insects may enter the mill from outside sources of infestation. Under such conditions genetic dilution could occur and thereby prevent the establishment of a homogeneous population.

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New Insecticides for Lygus Bug Control on Vegetable Seed Crops[1]

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ABSTRACT

Several new pesticides and combinations thereof were applied to table beet seed plants and to carrot seed plants in performance trials against the lygus bug, *Lygus hesperus* Knight. Six pesticides were tested: DDT, ethion, Dylox® (dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate), Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide), Trithion® (*S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate), and Di-Syston® (*O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorodithioate). These were used alone or in combinations. The natural population in small plots was augmented by three introductions resulting in an accumulated high of 23.5 bugs per plant during the tests. The trials on beets involved single sprays for eight plots; and a series of five applications for two plots.

A spray containing DDT plus Dylox gave the most complete control of lygus bugs, or 78% control during a 28-day period. Thiodan plus Dylox was almost equally good. Five sprays of Di-Syston and five dustings with DDT plus Trithion, and single sprays of DDT plus Trithion or DDT alone ranked in the mid-range of observed values, or 56% or 62% control. Thiodan or ethion, alone or in combination, were the least effective.

All applications containing Thiodan as well as the one containing DDT plus Dylox were appreciably more toxic to the pirate bug, *Orius tristicolor* White, than the other test materials. DDT plus Dylox spray had no perceptible effect on ladybeetles, *Hippodamia* spp. but sprays containing Di-Syston (5 applications) or ethion drastically reduced their numbers. DDT plus Trithion dust (5 applications) resulted in a significant increase in numbers of aphids and ladybeetles. The five Di-Syston sprays afforded a significant reduction in the number of plants infected by beet mosaic, and reduced the grade of infection.

The best treatments for control of lygus bugs also increased the average size and viability of beet seed. All but one of the treatments increased the average yield of seed per plant.

Dusts applied twice by aircraft to control lygus bugs in seed

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carrots were, in descending order of value for lygus bug control: 10% DDT plus 5% Dylox, 3% Thiodan, 10% DDT plus 2% Trithion, and 10% DDT. Seed yield was significantly increased in third-stage seed heads by the DDT plus Trithion dust. Increased yield for fourth-stage seed heads was obtained only from plants dusted with DDT plus Dylox, the only treatment that gave very significant increases in seed size for seeds threshed from third- and fourth-stage seed heads.

Lygus bugs are very difficult to control in many areas of California, especially so in certain vegetable and field seed crops. The difficulties relate to dense bug populations, large acreages, lack of uniformity in area controls, erratic selection of pesticides, and variations in the susceptibility of the bugs to each of the pesticides employed. Many new insecticides and combinations thereof have already been tested as possible substitutes for DDT or toxaphene and mixtures of these (Carlson 1959a). Bacon (1959) recently reviewed the problem of controlling this pest on alfalfa grown for seed and described results obtained with a number of new insecticides. Carlson (1959b) also has presented performance data on pesticides applied to table beet seed crops for lygus bug control.

This report covers trials of additional new pesticides on table beet and carrot seed crops, at Davis and Clarksburg, California. Incidental observations on side-effects of the treatments are included, *viz.*, effects on the crop plants and on other prevalent organisms such as aphids, spider mites, and predators.

LYGUS BUG CONTROL EXPERIMENTS ON TABLE BEET SEED PLANTS.—*Procedure.*—One part of the work was conducted in small replicated plots each consisting of four rows 25 feet long of beet seed plants grown at Davis, California. All plots were separated by two buffer rows. The beets (Detroit Dark Red, Improved) were directly seeded during September, 1958. This planting bolted in May, 1959. Plots established in this planting were manually sprayed for lygus bug control on June 5, 1959. Each treatment was replicated five times. The natural population of the lygus bug, *Lygus hesperus* Knight, within this planting was augmented by artificially introducing additional bugs at three intervals prior to beginning the experiment proper. Posttreatment counts of the plant bugs were made on 10 plants in each plot. The bugs were shaken from the plants, recovered, and counted as described elsewhere (Carlson 1956, 1959a). However, a larger aluminum funnel (15″ diameter) was used, so that three-quarters of the seed spikes per plant could be shaken into the funnel. Estimates of seed yield, seed size, and viability were based on samples of 20 plants in each of the plots.

The treatments listed in tables 1, 2, and 3 were applied when counts showed that practically all plants were in the stage of 53.7% bloom. Materials and formulations tested alone or in combinations were as follows: DDT, ethion, Dylox® (dimethyl 2,2,2,-trichloro-1-hydroxyethylphosphonate), Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide), Trithion® (*S*-(*p*-chlorophenylthio) methyl *O,O*-diethyl phosphorodithioate), and Di-Syston® (*O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorodithioate). Colloidal X-77 (an adjuvant) was added at the rate of 4 ounces per 100 gallons. Two of the plots received four additional applications prior to the spray timed especially to control lygus bugs. The Di-Syston sprays and DDT plus Trithion dusts were applied twice during the fall of 1958 and twice during the spring of 1959. This sequence of applications was made in order to obtain data on control of aphids and beet mosaic.

Results.—The counts and mortality data given in table 1 show that the most complete control of *L. hesperus* was given by the first two treatments listed. The lowest counts of both adults alone and adults plus nymphs were obtained in the plots sprayed once with DDT plus Dylox. The plots that were sprayed with Thiodan plus Dylox gave the next lowest counts in both categories. The counts

Table 1.—Mortality of lygus bugs during the first 28 days after application of test materials to table beet seed plants, Davis, California, 1959.

Treatments and Amounts (Active) per Acre	Average Mortality (%)[a] Adults Plus Nymphs					Average Number of Lygus Bugs per Replicate[b]		Final Control (%)
	Days after Treatment							
	3	7	14	21	28	Adults +Nymphs	Adults Only	Adults +Nymphs
DDT, 1 lb.+Dylox, 1 lb.	83	90	81	77	65	192.6 a	31.8 a	78
Thiodan, 1 lb.+Dylox, 1 lb.	85	88	80	72	61	215.6 ab	38.0 ab	76
Di-Syston, 2 lbs. (5 applications)[c]	83	89	71	62	23	334.6 c	38.8 ab	62
DDT, 10%+Trithion, 4% dust (5 applications)[c]	71	83	68	62	40	356.6 cd	59.2 bcd	60
DDT, 1 lb.+Trithion, 1 lb.	85	82	54	53	36	382.4 cd	45.2 abc	57
DDT, 1 lb.	78	68	58	49	43	393.8 cd	38.2 ab	56
Thiodan, 1 lb.+Ethion, 1 lb.	79	74	60	41	13	459.8 de	63.4 cd	48
Thiodan, 1 lb.+Trithion, 1 lb.	81	72	42	37	17	503.8 ef	61.0 cd	43
Thiodan, 1 lb.	80	52	36	36	29	531.0 efg	50.0 abc	40
Ethion, 1 lb.	78	46	24	31	8	629.4 gh	65.2 cd	29
Untreated	—	—	—	—	—	891.0 i	80.0 d	—

[a] Computations based on Abbott's formula. Each percentage value derived from bug counts averaged for 5 replications.

[b] Values under this heading are bug counts summed for 5 successive samplings and averaged for 5 replications. Significant differences in this and subsequent tables were computed according to Duncan's Multiple Range Test (1955). Significant differences between means (5%) are indicated when compared values have no letters in common.

[c] The five applications were for possible aphid control and reduction of beet mosaic. All of the other treatments were single spray applications of 50 gallons per acre. Each dust application was made by hand rotary duster at 40 to 45 pounds per acre.

Table 2.—Effect of insecticides on pirate bugs, ladybeetles, and aphids during a 28-day period after treatment of table beet seed plants for control of lygus bugs, Davis, California, 1959.

Treatments and Amounts (Active) per Acre	Pirate Bugs		Ladybeetles		Aphids
	Average No. Adults+Nymphs per Replicate	Mortality (%)	Average No. Adults+Larvae per Replicate	Mortality (%)	Average Number per Replicate
Thiodan, 1 lb.+Dylox, 1 lb.	71.8 a	74	8.0 bc	18	178 a
Thiodan, 1 lb.+Ethion, 1 lb.	78.4 ab	72	4.8 abc	51	13 a
DDT, 1 lb.+Dylox, 1 lb.	84.6 abc	69	10.8 c	0	167 a
Thiodan, 1 lb.	92.0 abc	67	6.0 abc	39	17 a
Thiodan, 1 lb.+Trithion, 1 lb.	100.8 bdc	64	5.8 abc	41	28 a
Ethion, 1 lb.	110.0 cde	60	4.2 ab	57	20 a
Di-Syston, 2 lbs. (5 applications)	140.8 f	50	2.8 a	71	10 a
DDT, 10%+Trithion, 4% dust (5 applications)	176.6 g	36	37.0 d	0	4,260 b
DDT, 1 lb.+Trithion, 1 lb.	209.8 h	24	8.4 bc	14	71 a
DDT, 1 lb.	247.0 i	11	9.2 bc	6	138 a
Untreated	277.2 j	—	9.8 c	—	27 a

recorded for these two treatments were not significantly different from each other, but both were significantly different from others in the series with respect to adult plus nymph counts. All of the listed treatments significantly decreased the numbers of lygus bugs appreciably below the numbers recovered from the untreated plots.

Since the lygus bug population averaged 23.5 bugs per plant, 75% control for the 28-day period of observation was considered to be good. Values in the range of 55% to 62% were called fair; those below 50% were judged to be poor.

When mortality percentages for adults plus nymphs are computed for each of the periodic counts, at 3, 7, 14, 21, and 28 days, control began to diminish rapidly after the 14th day in most of the plots. It is probable that second applications, 12 to 14 days after the first, would have been desirable in order to obtain good commercial control. The same data also reveal that DDT plus Dylox not only gave a high initial kill, but the residual effect of the mixture continued against all stages of the bugs for a longer period than the other pesticides tested.

The effects of these treatments on a minute pirate bug, *Orius tristicolor* White, are indicated in table 2. Thiodan plus Dylox appeared to be the most toxic treatment for these predaceous bugs. However, it was not significantly more toxic than Thiodan plus ethion, DDT plus Dylox, or Thiodan alone. All of these were significantly more toxic than the other treatments. No serious buildup of spider mites occurred in any of the plots. DDT was least toxic to pirate bugs, with an overall mortality of only 10.8%.

Data given in table 2 indicate that five applications of Di-Syston were very toxic to ladybeetles, *Hippodamia* spp. Ethion ranked next in toxicity to these beneficial insects, and both treatments were significantly more toxic than DDT plus Dylox. The great increase in ladybeetles induced by five applications of DDT plus Trithion dust was related to these factors: a correspondingly great increase in numbers of aphids, and to the low order of toxicity of the mixture to these predators. Unusually large numbers of the aphids, *Myzus persicae* (Sulz.) and *Aphis fabae* Scop.,[3] occurred only in this treatment. This occurrence was probably caused by the adverse effects of the dusts applied prior to the definitive application for lygus bugs. Conversely, there was no significant reduction in numbers of aphids by any of the treatments. How-

[3] Determined by R. C. Dickson, Department of Entomology, University of California Citrus Experiment Station, Riverside.

Table 3.—Effect of treatments for lygus bugs on weight and viability of beet seeds, and on average yield per beet seed plant, Davis, California, 1959.

Treatments and Amounts (Active per Acre)	Average			
	Weight 100 Seed Balls[a] (gm.)	Number Viable Seed Balls per 100	Number Germs per 100 (1–5 per Seed)	Seed Yield per Plant (gm.)
DDT, 1 lb.+Dylox, 1 lb.	1.84 a	90.4 a	155.6 a	53.8 c
Thiodan, 1 lb.	1.70 ab	79.2 bcd	130.2 d	52.3 c
Di-Syston, 2 lbs. (5 applications)	1.64 bc	88.2 ab	142.4 bc	55.3 a
DDT, 1 lb.	1.62 bcd	82.4 abc	138.6 bc	52.1 c
Thiodan, 1 lb.+Dylox, 1 lb.	1.62 bcd	80.2 bcd	125.6 de	53.8 c
Thiodan, 1 lb.+Trithion, 1 lb.	1.60 bcd	78.4 cd	123.0 ef	47.8 ac
DDT, 1 lb.+Trithion, 1 lb.	1.59 bcde	80.6 bcd	124.2 def	52.7 c
Ethion, 1 lb.	1.46 def	71.8 d	107.0 h	52.7 c
Thiodan, 1 lb.+Ethion, 1 lb.	1.41 f	78.6 cd	118.6 fg	52.2 c
DDT, 10%+Trithion, 4% dust (5 applications)	1.24 f	74.2 cd	116.0 gh	32.9 b
Untreated	1.37 f	74.8 cd	116.6 fg	44.8 c

[a] A good example of the influence of the weight of seeds on the size of seeds is shown by the number of seeds per ounce: 1543 for DDT plus Dylox and 2073 for the control.

Table 4.—Mortality of lygus bugs and minute pirate bugs during a 51-day period after dusting carrot seed plants by aircraft, Clarksburg, California, 1959.[a]

Treatments and Concentrations	Lygus Bugs			Minute Pirate Bugs	
	Average Number Adults	Average Number Adults+Nymphs	Final Mortality A+N (%)	Average Number Adults+Nymphs	Final Mortality A+N (%)
DDT, 10%+Dylox, 5%	5.3 a	20.2 a	85	66.7 ab	60
Thiodan, 3%	6.0 a	25.0 a	82	55.0 a	67
DDT, 10%+Trithion, 2%	5.8 a	29.2 a	79	72.1 ab	57
DDT, 10%	15.5 b	57.5 b	58	80.8 b	52
Untreated	39.8 c	136.8 c	—	167.8 c	—

[a] Values given as averages are bug counts summed for eight successive samplings and averaged for six subsamples taken for each large plot.

ever, five applications of Di-Syston spray resulted in a significant reduction of the number of plants infected by beet mosaic. The treated plot showed 10.4% infected plants whereas plants in the control plot showed 22.4% infection. This treatment also significantly reduced the index of severity of the mosaic infection from 0.91 to 0.34.

Seeds of greater average weight were harvested from the plot sprayed with DDT plus Dylox (table 3). The weight of 100 seeds was significantly greater than corresponding weights obtained from all other plots, except the ones sprayed with Thiodan. The beets sprayed with ethion alone or with Thiodan plus ethion did not produce heavier seeds than the untreated plants. Seeds of lowest average weight were harvested from the plots that received five dustings of DDT plus Trithion. Reduction of seed weight in this instance may have been related to the buildup of aphids in the plot.

All but one of the treatments increased average yields of seed but the amount of gain over the control was significant at the 5% level only for those plants sprayed five times with Di-Syston. The exception was the five-application treatment of DDT plus Trithion dust, which resulted in a significant reduction in yield.

Seeds harvested from the beet plants treated for lygus bugs were also tested for viability (table 3). The best of the treatments for lygus bug control greatly increased seed germination. For example, seeds harvested from the plants sprayed with DDT plus Dylox produced the greatest number of germs per seed ball. This number was significantly higher than that obtained from plants otherwise treated. This treatment also increased the number of germinating seed balls. Most of the other treatments increased seed viability to some extent, whereas ethion spray reduced seed viability appreciably below values obtained for the control.

Table 5.—Effect of aircraft dust treatments for lygus bugs on weight of carrot seeds, and on yield per seed head, Clarksburg, California, 1959.

Treatments and Concentrations	Average Weight of 100 Seeds in Grams for 2 Stages of Seed Heads[a]		Average Yield in Grams per Seed Head for 2 Stages of Seed Heads	
	3rd Stage	4th Stage	3rd Stage	4th Stage
DDT, 10%+Dylox, 5%	0.141 a	0.146 a	1.75 b	0.076 a
DDT, 10%+Trithion, 2%	.116 bc	.122 b	2.19 a	.053 b
DDT, 10%	.121 b	.126 b	1.68 b	.034 cd
Thiodan, 3%	.110 c	.106 d	1.77 b	.041 bc
Untreated	.120 b	.117 c	1.49 c	.029 cd

[a] A good example of the influence of the weight of seeds on the size of seeds is shown by the number of seeds per ounce for some treatments sampled at the fourth stage: 19,452 for DDT plus Dylox, 24,275 for the control, and 26,792 for the Thiodan treatment.

There were no significant increases in weight or yield of seeds for seed heads of the first and second stages.

Control of Lygus Bugs on Carrot Seed Plants with Dusts Applied by Aircraft.—*Procedure.*—The second part of this work concerns lygus bug control on carrot seed plants with various dusts applied by aircraft. Red Core Chantenay carrots growing near Clarksburg, California were divided into 4 plots of 6 acres each. A small area of 1½ acres was retained as a control plot. Dust materials as listed in tables 4, 5 and 6 were applied twice, each time at 35 to 40 pounds per acre. The first dusting was made on May 24 and the second on June 10, 1959.

Eight samplings for numbers of plant bugs were made at intervals of about 7 days, beginning on the 3rd day after the first dusting and continuing thereafter until the 51st day. Counts were made for six separate areas within each plot on every sampling date. Each of these was made by shaking 10 seed heads over a funnel fastened to the lid of a quart jar.

Yield data were obtained from plants from five locations per plot. The sizes of the subsamples were varied according to stage of seed head growth, *viz.*, 10 heads of the first stage, 20 heads of the second, 30 of the third, and 40 of the fourth. Thus, the numbers of seed heads examined were increased as their sizes diminished through each of the growth stages.

Results.—The effects of treatments on populations of lygus bugs and minute pirate bugs are shown in table 4. A fairly damaging but not severe infestation of lygus bugs developed during the course of these investigations. The series of eight samplings within the control plot yielded a cumulative average of 1.7 bugs per plant.

As judged on the basis of counts of lygus adults plus nymphs, the first three treatments listed in table 4 were not distinguishable from each other. These were significantly better than the plain DDT dusts. The numbers of

Table 6.—Effect of aircraft dust treatments for lygus bugs on the viability of carrot seeds, Clarksburg, California, 1959.

Treatments and Concentrations	Average Viability of 100 Seeds for Four Stages of Seed Heads			
	First	Second	Third	Fourth[a]
DDT, 10%+Dylox, 5%	93.2 a	92.2 a	80.2 ab	60.4 o
DDT, 10%+Trithion, 2%	93.4 a	91.2 a	86.2 a	59.0 o
Thiodan, 3%	92.6 a	94.0 a	84.4 a	57.4 o
DDT, 10%	88.6 a	88.8 a	79.2 ab	55.4 o
Untreated	80.4 b	76.0 b	61.8 c	48.0 o

[a] The viability of seeds of the fourth stage seed heads was not significantly different among treatments because of extreme variability in germination.

pirate bugs were appreciably reduced by each of the treatments. The anthocorids were most susceptible to Thiodan as used in this trial; they were least affected by DDT. Spider mites did not appear in any of the plots.

Interpretable results on carrot seed yield are generally difficult to obtain in this kind of experimentation. In this instance, the yields from first- and second-stage seed heads were not significantly better than the yield from the control plot. Significantly increased yields for third-stage heads were obtained with DDT plus Trithion (table 5). DDT plus Dylox produced the best yield for the fourth-stage heads. Only two of the four treatments, DDT plus Dylox and DDT plus Trithion, resulted in higher yields than obtained from the control. It appeared that neither DDT nor Thiodan dusts gave good enough control to protect seed heads of the fourth-stage.

DDT plus Dylox was the only one of the treatments to give an increase in the weight of 100 seeds, as determined for third- and fourth-stage seed heads (table 5).

Seed viability appeared to be superior for seed heads of the first two stages (table 6). All of the treatments increased germination of seeds harvested from first- and second-stage heads. The several treatments could not be differentiated from each other in this respect, but all were significantly better than the control. Increased seed germination was less pronounced in the case of the third-stage seed heads, and was not different from the control for seeds of the fourth-stage heads.

DISCUSSION.—Investigations by Carlson (1959a,b) and Bacon (1959) demonstrated that Dylox is promising for lygus bug control on vegetable or field seed crops. While this pesticide is known to have a number of desirable attributes, it does not persist long enough to protect seed crops against these insects. Results obtained in this investigation, however, further confirm the observation that a combination of Dylox with DDT is especially effective for use on vegetable seed crops. It has been demonstrated that combinations of Dylox and other materials, such as toxaphene and Thiodan, also give satisfactory reductions of lygus bugs, but they are more toxic to beneficial insects.

The optimum dosage of DDT plus Dylox for lygus bug control is believed to be 1 pound of each (active) per acre for carrot seed and table beet seed crops. The necessity of thorough and timely insecticidal applications has also been demonstrated. During 1959 an application of DDT dust made late in the morning to an adjacent carrot seed acreage resulted in unsatisfactory control, and resulted in a 10-day average count of 7.1 bugs per sample, compared with 2.9 bugs per sample for an early morning application.

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The Effect of Soil Type and Moisture on Germination and Growth from Wheat Seed Treated with Phorate[1]

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ABSTRACT

Studies were conducted in the greenhouse to determine the effect of soil type and moisture on the viability of phorate-treated wheat seed. The effects of different soil types and different moisture levels on germination and early growth of wheat were found to be highly significant. Seed viability was adversely affected by excess moisture, regardless of treatment. Although germination under minimum moisture conditions was only slightly impaired, plant growth was severely reduced. It was found that soils and moisture levels may interact in their effect on seed viability. Several interactions were highly significant. The clay silt-loam and the muck soil gave opposite results under conditions of minimum moisture. With minimum watering, relatively high germination and yield were obtained in muck in contrast with low germination and low yield in clay silt-loam. With excess moisture, germination was low in both soils.

Untreated seed exhibited low viability in muck soil, probably owing to soil microörganisms, as germination was substantially improved by the addition of a fungicide. Germination of seed treated with the highest rate of phorate, 0.5 pounds per 100 pounds of seed, was relatively high in the very fertile soils (muck and Agronomy Farm silt-loam). This suggests an apparent tying up of the insecticide by the organic matter in the soil. In general, phorate-treated seed was less viable in soils having a low water-holding capacity. Viability of phorate-treated seed was also less with moisture factors which tended to delay germination, whether due to low or excessive amounts of water.

An increasing need for fall forage has prompted investigators to examine the possibility of early fall seeding of wheat in order to utilize the crop for fall and spring pasture. Patterson (1959) found that early seeding of wheat in the fall significantly increased the yield. Wilson *et al.* (1960) indicated that treatment of wheat seed with phorate increased yield, even when the crop was clipped to simulate grazing.

However, several investigators had found that phorate

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was phytotoxic to the seed and severely reduced germination (Skogg 1959, Guyer *et al.* 1958, Wilson *et al.* 1960). Previous work by Kirk & Wilson (1960) indicated that much of the loss from phorate could be alleviated by the addition of a suitable combination of sticker and fungicide.

Other ecological factors have been suspected of affecting germination of crops in which the seed was treated with phorate. Reynolds *et al.* (1957) tested phorate and Di-Syston® (*O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorodithioate) as seed treatments on alfalfa, sugar beets, and cotton. They recorded no appreciable loss of stand at the rate of 1 pound of toxicant per 25 pounds of seed with both materials. They did report, however, that soybean and sorghum seed was badly impaired with both insecticides. Di-Syston was less damaging than phorate. It was concluded that phytotoxicity from these insecticides varies with different seed and under different conditions.

Skogg (1959) mentioned that cool wet conditions adversely affected germination of wheat treated with phorate. Several workers (Parencia *et al.* 1957, Hanna 1958, Dobson 1958) reported loss in stands of cotton from seed treated with phorate and Di-Syston especially when cool, wet conditions prevailed after planting. The latter two workers stated, however, that this resulted in no significant loss in yield. Steudel (1958), in testing systemic insecticides for aphid control in Germany, recorded phytotoxic conditions in dry soil.

Studies reported in this paper were undertaken to determine the effects of moisture and soil type on the germination, growth, and forage yield of wheat which had been seed-treated with phorate.

METHODS AND MATERIALS.—All experiments were carried out in the greenhouse at a temperature ranging from 70° to 85° F., using the variety, LaPorte. Phorate was applied to the seed by Mr. W. B. Gehl of the American Cyanamid Company in the form of Thimet 44-D, in which the active material is impregnated on activated charcoal. Rates of 0, 0.25, and 0.50 pounds of active phorate were used per 100 pounds of seed. Captan, a fungicide, was first applied to the seed at the rate of 3 ounces per 100 pounds of seed. A sticker used to adhere the toxicant to the seed consisted of 3.0 parts dextrin, 1.5 parts sorbitol, and 0.5 parts water.

Six soils described in table 1 were utilized in this study with three levels of moisture (minimum, adequate, and excessive). Treatments were replicated four times using a single 4-inch "clay" pot per replicate in which 25 seeds were planted. The experiment was designed as a complete factorial test. Treatments receiving minimum moisture were watered slightly to initiate germination, and thereafter only enough to maintain the plants in a slightly wilted condition. Adequate moisture was maintained by adding sufficient water daily to keep the plants growing vigorously. This amount varied with conditions in the greenhouse. Treatments receiving excess moisture were placed in flats lined with polyethylene film. These were filled with sufficient water to immerse the pots to one-half their depth. Germination and plant weights were recorded at 14 days.

Table 2.—Per cent germination of wheat seed treated with phorate and planted in six different soils at three moisture levels.

Soil[a]	Moisture Level	Treatment				
		Controls			Lbs. Phorate/100 Lbs. Seed	
		Untreated	Captan	Captan + Sticker	0.25	0.50
1	Minimum	83	93	91	81	78
	Adequate	86	90	92	84	82
	Excessive	53	49	52	16	14
2	Minimum	85	91	96	86	75
	Adequate	92	93	91	87	80
	Excessive	47	29	27	16	6
3	Minimum	84	86	93	70	54
	Adequate	90	85	88	86	73
	Excessive	54	69	71	30	24
4	Minimum	86	95	92	93	88
	Adequate	86	93	91	86	81
	Excessive	3	25	4	8	4
5	Minimum	89	88	87	79	72
	Adequate	85	94	88	83	82
	Excessive	19	32	33	10	2
6	Minimum	89	81	91	83	81
	Adequate	94	91	89	82	82
	Excessive	0	4	0	0	1

[a] 1. Silt loam of high fertility; 2. Silt loam of low fertility; 3. Clay-silt loam of high fertility; 4. Muck; 5. Loam of high fertility; 6. Sand.

RESULTS AND CONCLUSIONS.—Germination data for all treatments are presented in table 2. Analysis of the data showed that differences in germination and plant growth were highly significant between soils, between moisture levels, and between treatments. Interactions were found between soils and moisture levels (table 3), between soils and treatments (table 4), and between treatments and moisture levels for germination (table 5). For forage weight, soils×moisture levels and treatments ×moisture levels had significant interactions (tables 6 and 7). Examination of the data also suggests that a three-way interaction occurred between soils, moisture level, and treatments on yield (table 3).

These studies confirmed previous results reported by

Table 1.—Characteristics of soil types used in experiments.

Soil No.	Texture	Locality Collected	pH	Color	Pounds/Acre	
					P_2O_5	K_2O
1	Silt loam	Purdue Agronomy Farm, Lafayette, Indiana	5.9	Dark brown	485	238
2	Silt loam	Brownstown, Illinois	5.0	Light yellowish brown	35	144
3	Clay silt loam	Herbert Davis Farm (Purdue), Farmland, Indiana	5.8	Light brown	191	173
4	Sandy organic (muck)	Romney, Indiana	7.1	Black	860	169
5	Loam	Pinney-Purdue Farm, Wanatah, Indiana	5.3	Brown	455	313
6	Sand	Lafayette, Indiana	7.0	Light yellowish brown	510	140

Table 3.—The influence of soil type and moisture level on the per cent germination of wheat seed.

Moisture Level	Soil[a] 1	2	3	4	5	6	Moisture Level Means
Minimum	85.2	86.6	77.4	90.8	83.0	85.0	84.7
Adequate	86.8	88.6	84.4	87.4	86.4	87.6	86.9
Excessive	36.8	25.0	49.6	8.8	19.2	1.0	23.4
Soil means	69.6	66.7	70.5	62.3	62.9	57.9	

L.S.D.	5%	1%
Soil means	1.1	1.4
Moisture level means	.8	1.0
Within soils	1.9	2.5
Within moisture levels	1.9	2.5

[a] 1. Silt loam of high fertility; 2. Silt loam of low fertility; 3. Clay-silt loam of high fertility; 4. Muck; 5. Loam of high fertility; 6. Sand.

Table 4.—The influence of soil type on the per cent germination of phorate-treated wheat seed.

Soil[a]	Treatment: Controls Untreated	Captan	Captan +Sticker	Lbs. Phorate/100 Lbs. Seed 0.25	0.50	Soil Means
1	74.0	77.3	78.3	60.3	58.0	69.6
2	74.7	71.0	71.3	63.0	53.7	66.7
3	76.0	80.0	84.0	62.0	50.3	70.5
4	58.3	71.0	62.3	62.3	57.7	62.3
5	64.3	71.3	69.3	57.3	52.2	62.9
6	61.0	58.7	60.0	55.0	54.7	57.9
Treatment means	68.1	71.6	70.9	60.0	54.4	

L.S.D.	5%	1%
Soil means	1.1	1.4
Treatment means	1.0	1.3
Within soils	2.5	3.2
Within treatments	2.5	3.2

[a] 1. Silt loam of high fertility; 2. Silt loam of low fertility; 3. Clay-silt loam of high fertility; 4. Muck; 5. Loam of high fertility; 6. Sand.

Table 5.—Per cent germination of phorate-treated wheat seed as influenced by moisture.

Moisture Level	Treatment: Controls Untreated	Captan	Captan +Sticker	Lbs. Phorate/100 Lbs. Seed 0.25	0.50	Moisture Level Means
Minimum	86.0	89.0	91.7	82.0	74.7	84.7
Adequate	88.8	91.0	89.8	84.7	80.0	86.9
Excessive	29.3	34.7	31.2	13.3	8.5	23.4
Treatment means	68.1	71.6	70.9	60.0	54.4	

L.S.D.	5%	1%
Treatment means	1.0	1.3
Moisture level means	.8	1.0
Within treatments	1.7	2.3
Within moisture levels	1.7	2.3

Table 6.—The influence of soil type and moisture level on forage yield (mgs.) of wheat grown in greenhouse.

Moisture Level	Soils[a] 1	2	3	4	5	6	Moisture Level Means
Minimum	6.66	6.52	5.30	7.24	4.46	5.38	5.93
Adequate	14.76	13.56	15.06	19.48	18.50	15.48	16.14
Excessive	8.1	5.84	14.24	3.16	3.08	0.22	5.77
Soil means	9.84	8.64	11.53	9.96	8.68	7.03	

L.S.D.	5%	1%
Soil means	0.36	0.48
Moisture level means	.25	.34
Within soils	.63	.83
Within moisture levels	.63	.83

[a] 1. Silt loam of high fertility; 2. Silt loam of low fertility; 3. Clay-silt loam of high fertility; 4. Muck; 5. Loam of high fertility; 6. Sand.

Table 7.—Yield of forage (mgs.) from phorate-treated wheat seed under different moisture levels.

Moisture Level	Treatment: Controls Untreated	Captan	Captan +Sticker	Lbs. Phorate/100 Lbs. Seed 0.25	0.50	Moisture Level Means
Minimum	5.72	6.93	7.35	5.78	3.85	5.93
Adequate	15.45	16.80	17.63	16.17	14.65	16.14
Excessive	6.98	8.90	8.90	2.68	1.40	5.77
Treatment Means	9.38	10.88	11.29	8.21	6.60	

L.S.D.	5%	1%
Treatment means	0.33	0.43
Moisture level means	0.25	0.34
Within treatments	0.58	0.76
Within moisture levels	0.58	0.76

Kirk & Wilson (1960) showing that germination declined as the dosage of phorate was increased, and that some of the loss can be counteracted by the addition of a fungicide. This occurred throughout with the exception of soil 2, a highly acid silt-loam of low fertility, and soil 6, a sand (table 4). Forage yield also followed these trends, probably as a direct result of the effect on germination.

Germination was consistently low in treatments with excess moisture. Interactions, with opposite effects, apparently occurred between soils and moisture. With low moisture, germination and forage yield in muck were relatively high. In the clay silt-loam under the same conditions, both germination and yield, in contrast, were low. This was apparently owing to the vast water-holding capacity of muck, and the quick-drying properties of the clay silt-loam.

Interactions also occurred between muck and the various treatments (table 4). Untreated seed showed significantly lower viability in muck soil, which was probably owing to soil pathogenic microörganisms present in that type of soil, as germination was vastly increased when a fungicide was used. It is of particular interest that when phorate was applied to the seed, the germination was highest in the muck soil and Agronomy Farm soil, both of which have a high organic matter content.

The results of these studies indicate that soil type is of considerable importance in modifying the phytotoxic effects of phorate when applied to wheat seed. This probably may be attributed to the apparent property of an organic soil to tie up an organic insecticide so that it is unavailable to the seed or seedling. Similar results have been reported by Lichtenstein & Schultz (1959). They found that certain chlorinated hydrocarbons were more persistent in muck than in Miami silt-loam, suggesting that the insecticide was bound to the organic portion of the soil. Germination of phorate-treated seed was relatively good in sand under conditions of minimum and adequate moisture, but with excess moisture, there was nearly a complete lack of viability in the seed (table 3).

It is suggested that phorate phytotoxicity will generally be of lesser consequence in highly organic soils, since these soils normally possess qualities which induce rapid germination and growth. Conditions which tend to delay germination of phorate-treated seed appear to increase phytotoxicity.

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Feed Additives for Control of House Fly Larvae in Livestock Feces[1]

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ABSTRACT

There was no reduction in the numbers of house flies (*Musca domestica* L.) completing development from egg to adult in feces from a steer fed up to 100 gm./day of Polybor 3, (containing 98% of disodium octoborate tetrahydrate ($Na_2B_8O_{13}-4H_2O$)). When mixed directly with cattle manure, Polybor 3 was an effective larvicide; a possible explanation for relatively higher rates in a steer ration not controlling larvae in feces is discussed. *Bacillus thuringiensis* Berliner spores containing 25 billion/gm. mixed directly with cattle feces at rates of 0, 50, 100, 200, and 300 mg./kg. and inoculated with 100 house fly eggs resulted in an average of 43, 41, 9, 5, and 0 adult house flies completing development, respectively. Feeding 20 gm. *B. thuringiensis*/day to a steer prevented development of house flies in manure, and 0.125 gm. *B. thuringiensis*/kg. ration greatly reduced the number of adult flies emerging from hen droppings. The presence of aureomycin did not decrease the effectiveness of this pathogen.

Adding substances to livestock feed to pass in feces and render them toxic to fly larvae has been considered by several investigators. Bruce (1939) reported that first attempts to control horn flies (*Siphona irritans* (L.)) in this manner by Gallagher (1928) were unsuccessful. Knipling (1938) and Bruce (1939) prevented development of horn fly larvae in cattle feces by feeding phenothiazine. Bruce (1940) indicated that rotenone fed to cattle daily at 0.3 gram/100 pounds of body weight controlled horn fly larvae. More recently Polybor 3 (containing 98% of disodium octoborate tetrahydrate ($Na_2B_8O_{12}-4H_2O$)) fed to caged layers resulted in control of house fly (*Musca domestica* L.) larvae in droppings (Burns *et al.* 1959). Similar control was reported from feeding *Bacillus thuringiensis* Berliner to caged layers (Briggs 1959) and to steers (Dunn 1959). *B. thuringiensis* was widely tested as an insect pathogen, especially for control of lepidopterous larvae (Steinhaus 1951, Hall & Dunn 1958).

This investigation was to test Polybor 3 as an additive to a steer ration for control of house fly larvae in feces and to verify the results of Briggs and Dunn with *B. thuringiensis*.

MATERIALS AND METHODS.—*Polybor 3*[3]—Five kilograms of feces were collected from grain-fed steers, mixed and divided into 1-kg. samples. Each of the five samples was treated with Polybor 3 at one of the following rates: 0, 0.5, 1, 2, and 4 gm. Then each sample, including the control, was divided into four 250-gm. subsamples. Each subsample was formed into a mass 4 inches in diameter, placed in a 6-inch pot saucer and inoculated with 100 house fly eggs. All flies used in this investigation were a laboratory strain, designated KUN, with no history of insecticide exposure. The eggs were individually transferred from oviposition pads to depressions in manure with a camel's-hair brush. Five ounces of dry sand placed around the perimeter of each manure sample served as an area for pupation. After inoculation with eggs, each sample was covered with wire screen until adult flies emerged and died. The dead adult flies were counted about 3 weeks after the samples were inoculated. Tests were conducted in a greenhouse at about 75° F.

Two yearling Hereford steers weighing about 700 pounds each were used to determine the efficacy of Polybor 3 incorporated in the ration as a house fly larvicide. On basis of results of the test in which Polybor 3 was mixed directly with manure, it was decided to feed four different levels of Polybor 3 (40, 60, 80, and 100 gm./head/day). The larvicide was fed with the daily ration of 10 lbs. sorghum grain, 15 lbs. sorgo silage, 3 lbs. chopped alfalfa hay, 1½ lbs. cottonseed meal and 72 mg. of aureomycin. Animals were housed in separate pens in an unheated building and fresh fecal samples were col-

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[3] Supplied by Southeastern Supply Company, Auburn, Ala.

lected from the floor. The experiment was conducted during the winter when no flies were present. After manure was collected from both steers at the beginning of the experiment, one steer received the four progressive levels of Polybor 3. Following a 5-day preliminary feeding period at each treatment level, fecal samples were collected. Feces were also obtained from the untreated steer at the same time. Five days after the 100-gm. feeding level was discontinued, a posttreatment sample was collected. Manure samples were stored at 0° C. until 1 week after the posttreatment collection. Then each sample was divided into six subsamples of 200 gm. each and tested using the technique previously described.

Bacillus thuringiensis[4]—The commercial preparation of *B. thuringiensis* used in these tests was rated at 25 billion spores per gram by Rohm & Haas Company. The methods used in testing the effect of spores added directly to manure were the same as previously described for Polybor 3 except that each of the five 1-kg. samples was treated with spores at one of the following rates: 0, 50, 100, 200, and 300 mg.

During November, 1959, eight Leghorn hens, out of production and each weighing about 5 pounds, were used to test ingested *B. thuringiensis* as a pathogen for house fly larvae. The test was conducted in an unheated building, with the hens suspended in individual wire cages over plastic sheets from which droppings were collected. On the basis of preliminary findings the spores were added at a rate of 0.125 gm./kg. of ration consisting of equal parts of cracked wheat and a commercial laying feed. Hens were fed a known quantity of feed, *ad lib.*, and orts were weighed back at the end of the experiment to determine daily intake. Four hens received the treated ration and four were given untreated feed for 18 days. After an 8-day preliminary feeding period, droppings were allowed to accumulate for 10 days and then tested by the procedure previously described for steer feces. Four replications of both the treated and untreated droppings were compared.

Methods used to evaluate *B. thuringiensis* in the steer ration as a pathogen for house fly larvae were essentially those described for Polybor 3 except a single rate was tested. Spores were included in the same ration previously described at the rate of 20 gm./head/day. Fecal samples were collected from treated and untreated steers on the fifth day after treatment was initiated.

To determine what effect aureomycin would have on *B. thuringiensis* and on house fly development, four identical samples of steer feces, each weighing 1 kg., were treated as follows: (1) 200 mg. of *B. thuringiensis*; (2) 330 mg. aureomycin; (3) 200 mg. of *B. thuringiensis* plus 330 mg. of aureomycin; and (4) no treatment. Each sample was divided into five 200-gm. subsamples each of which was inoculated with 100 house fly eggs and tested in the manner described previously.

Table 1.—Average number of house flies completing development (egg to adult) in cattle feces treated with Polybor 3 or *Bacillus thuringiensis* Berliner.

Polybor 3[a]		B. thuringiensis[b]	
Rate (gm./kg.)	Average No. Adults from 100 Eggs	Rate (mg./kg.)	Average No. Adults from 100 Eggs
0.5	51	50	41
1	2	100	9
2	0	200	5
4	0	300	0
Untreated	35	Untreated	43

[a] 98% disodium octoborate tetrahydrate ($Na_2B_8O_{13}$-$4H_2O$).
[b] 25 billion spores/gm.

Table 2.—Average number of house flies completing development (egg to adult) in manure collected from a steer fed Polybor 3 and an untreated steer.

Rate of Polybor 3[a] (gm./head/day)	Average No. Adults from 100 Eggs	
	Untreated	Treated
Pre-treatment	54.0	53.5
40	61.8	63.8
60	60.5	60.3
80	58.3	68.5
100	61.0	66.2
Post-treatment	56.3	63.7

[a] 98% disodium octoborate tetrahydrate ($Na_2B_8O_{13}$-$4H_2O$).

Table 3.—Average number of house flies completing development (egg to adult) in manure collected from hens and a steer fed *Bacillus thuringiensis* Berliner.

Animals	Rate of Treatment[a] (gm. of spores/head/day)	Average No. Adults from 100 Eggs		Control (%)
		Treated	Untreated	
Hens	0.0078	2.1	24.7	92
Steer	20	0.0	66.4	100

[a] 25 billion spores per gm.

RESULTS.—*Polybor 3.*—Control of house fly larvae was obtained in steer manure treated with Polybor 3 at one gm./kg. but not at half this rate as presented in table 1. Polybor 3 fed at rates up to 100 gm./head/day to a steer weighing about 700 lbs. resulted in no significant control of house fly larvae in manure, as shown in table 2. Feed intake tended to decrease several days after the steer was started on 80 and 100 gm. of Polybor 3/day.

Bacillus thuringiensis.—Spores mixed directly with steer feces at a rate of 300 mg./kg. prohibited fly development (egg to adult) and 100 mg./kg. reduced it significantly as shown in table 1. No effect on fly development was evident in manure treated at 50 mg./kg.

When hens were fed a ration treated with 0.125 gm. of *B. thuringiensis*/kg., the actual amount of spores consumed averaged 7.8 mg./hen daily. As shown in table 3 this prevented 92% of house flies from completing development in droppings. Average daily feed intake for hens was 62 gm./head of treated ration and 66 gm. of untreated ration.

Data presented in table 3 show that the development of house flies was prevented in feces from a steer fed 20 gm. of *B. thuringiensis* spores/day. Feed intake of this steer did not appear to be affected by including spores at this rate in the ration. Although the ration included 72 mg. of aureomycin/day, this did not nullify the effect

[4] Furnished by Rohm & Haas Company, Philadelphia 5, Pa.

Table 4.—Effect of aureomycin on *Bacillus thuringiensis* and on development of house flies (egg to adult) in cattle feces.

TREATMENT[a]	RATE[b] (MG./KG.)	AVERAGE NO. ADULTS FROM 100 EGGS
Aureomycin+ *B. thuringiensis*[c]	330 200	0.2
B. thuringiensis	200	0.2
Aureomycin	330	49.0
Untreated		38.2

[a] All manure samples collected from cattle receiving 72 mg. of aureomycin day.
[b] These amounts mixed directly into cattle feces.
[c] 25 billion spores/gm.

of *B. thuringiensis* on development of house flies in feces.

Data presented in table 4 further indicate that aureomycin did not decrease the pathogenicity of *B. thuringiensis* for house flies. Aureomycin alone seemed to increase the number of flies completing development. Although further testing is needed to verify this finding, the increase shown in table 4 approached significance at the 5% level.

DISCUSSION.—Since Polybor 3 fed to caged layers resulted in control of house flies in droppings (Burns *et al.* 1959), it is not fully understood why it gave no control when fed to a steer. A possible explanation may be found in differences in excretory systems between chickens and steers. Owen (1944) found that milk cows receiving oral doses of 16 to 20 gm. daily of borax eliminated 12 to 14 gm./day in urine and from 4 to 6 gm. in feces. Assuming Polybor 3 and borax are excreted similarly, it would be necessary to feed a higher rate in a steer ration to obtain the same concentration of larvicide in steer feces as in poultry droppings, which include both feces and urine. For this reason relatively higher rates were fed to the steer than were reported to be effective with poultry. However, because Polybor 3 is more soluble than borax, even a greater proportion of Polybor 3 may be eliminated in urine. So, the above compensation may not have been great enough. Lack of palatibility prevented greater amounts of Polybor 3 being fed to the steer. If solubility of these compounds affects their excretion patterns, a more insoluble borate might be more effective than Polybor 3 for use as a feed additive in a cattle ration to control house fly larvae.

Results with *B. thuringiensis* used as a feed additive agree with those of Briggs (1959) and Dunn (1959) in that the pathogen appeared effective in reducing numbers of house flies completing development in feces. It appeared in tests with *B. thuringiensis*, as reported by Dunn (1960), that many flies died during the pupal stage. High mortality in the pupal stage was also noted for *Aedes aegypti* (L.) by Liles & Dunn (1959). The spores, at the single rates tested, seemed acceptable to steers and hens when mixed in feed rations. Aureomycin, at rates normally administered to cattle, apparently would not reduce effectiveness of *B. thuringiensis* for house fly control.

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Studies on Resistance to Malathion in the Mosquito, *Culex tarsalis*[1]

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ABSTRACT

A colony of *Culex tarsalis* Coq., 60 times resistant to malathion was found to be resistant to malaoxon (*S*-[1,2-bis(ethoxycarbonyl)ethyl] *O,O*-dimethyl phosphorothiolate), and the diethyl homolog of malathion (*S*-[1,2-bis(ethoxycarbonyl)ethyl] *O,O*-diethyl phosphorodithioate), although to a lesser degree. Slight resistance to Co-Ral® (*O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate) was also observed, but no resistance was found to any other of a series of 14 organophosphorus insecticides. Two- to threefold resistance to DDT and dieldrin was also found. Larvae of the resistant and a susceptible colony degraded malathion at about the same rate and in the same manner, largely through the formation of carboxylic acid derivatives.

Resistance to malathion in the mosquito *Culex tarsalis* Coq. was first described from the Fresno, Calif., area by Gjullin & Isaak (1957). Since malathion is a relatively safe organophosphorus insecticide which is widely used in mosquito control, it seemed important to learn something of the nature of the mechanisms involved in this resistance. An investigation was therefore made on the extent of cross-resistance to other insecticides present in a malathion-resistant strain and on the rate and nature of the detoxification of malathion by larvae of a resistant and of a susceptible strain of *C. tarsalis*.

The most likely cause of resistance to phosphorus insecticides appears to be increased ability of the insect to detoxify the insecticide by hydrolysis of one or more of the several ester linkages to form nontoxic phosphoric acids. Malathion is of particular interest in this respect, since it possesses two carbethoxy ester bonds in addition to the three usual phosphorus ester groupings. There is considerable evidence to show that the major detoxification products to malathion in mammals are formed by hydrolysis at one or both of the carbethoxy ester bonds (March *et al.* 1956, Cook & Yip 1958, Seume & O'Brien 1960). It has been suggested that insects are more susceptible to malathion than warm-blooded animals because of their lesser ability to detoxify it in this manner (O'Brien 1957). More recently it has been shown that the house fly (*Musca domestica* L.) and American cockroach (*Periplaneta americana* (L.)) hydrolyze malathion relatively slowly and primarily at the phosphate ester bond, whereas the mouse hydrolyzes it rapidly and primarily at the carbethoxy ester bond (Krueger & O'Brien 1959).

TEST INSECTS.—A colony of malathion-resistant *Culex tarsalis* Coq. is maintained in our laboratory. This colony was obtained from the Fresno, California area in 1956 as described by Eddy (1958) and is about 60 times as resistant to malathion (based on LC-50 values obtained with fourth instar larvae) as a susceptible colony of the same species also reared here. At the time the malathion colony was obtained it also had about a fivefold resistance to DDT. Rearing methods have been described (Eddy *et al.* 1958).

The tolerance to malathion in the resistant colony is maintained by rountine pressuring of fourth-instar larvae and has remained at a nearly constant level for 2 years. For the sake of brevity, the malathion-resistant colony will hereafter be referred to as R and the susceptible colony as S.

COMPARATIVE BIOASSAY AND RESISTANCE.—*Procedure.*—The susceptibility of both colonies of mosquitoes to a series of organophosphorus insecticides and to DDT and dieldrin was determined. The compounds used represent most of the available types of phosphorus-containing insecticides. They were chosen so that their various structural similarities and dissimilarities to malathion might indicate which of the various groupings present in the malathion molecule is responsible for the resistance present.

All insecticide solutions were prepared in acetone immediately before use. With each compound tested, larvae of both colonies were bioassayed at the same time with the same insecticide solution to eliminate possible differences due to aging and decomposition of the test solutions. However, the materials were not all tested at the same time or on the same population so their LC-50 values are not directly comparable. The following compounds were tested: malathion (95%), demeton (technical), dimethoate (technical), Dipterex® (dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate)(technical), DDVP (technical), ronnel (technical), the *O*-analog of ronnel (dimethyl 2,4,5-trichlorophenyl phosphate) (purified), Phosdrin® (1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate) (technical), Dow ET-15® (*O*-(2,4,5-trichlorophenyl) *O*-methyl phosphoramidothiate) (technical), Delnav® (2,3-*p*-dioxanedithiol *S,S*-bis (*O,O*-diethyl phosphorodithioate) (technical), Co-Ral® (*O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate) (purified), para-oxon (purified), Chlorthion® (*O*-(3-chloro-4-nitrophenyl) *O,O*-dimethyl phosphorothioate) (90%), parathion (98.5%), EPN (technical), DDT (purified), and dieldrin (technical).

For bioassay, groups of 20 fourth-instar larvae were placed in 250 ml. of distilled water treated with 0.2 ml. of the insecticide in acetone solution. Mortalities were recorded 24 hours later and were based on whether or not a response was obtained when larvae were touched with a small wire. All tests were run in duplicate and replicated twice. In testing the large series of compounds described above, each material was used at concentrations of 1 p.p.m., 0.3 p.p.m., 0.1 p.p.m., etc. Compounds in this series were not tested in more detail since these concentrations of insecticide were considered adequate to show whether or not any differences in susceptibility were present.

As a next step, samples of a series of compounds more closely related structurally to malathion were bioassayed in more detail. These materials were tested as received

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[2] The following persons supplied some of the materials used in the study: R. D. O'Brien and W. C. Dauterman, University of Wisconsin, Madison, labeled malathion and labeled and nonlabeled acethion; B. W. Arthur, Alabama Polytechnic Institute, Auburn, dimethyl homolog of acethion; and R. W. Young and E. L. Clark, American Cyanamid Co., Stamford, Conn., malaoxon, diethyl homolog of malathion, and the mono- and di-carboxylic acid derivatives of malathion.

and were generally in a high degree of purity, having been purified by column chromatographic or vacuum distillation techniques. The compounds included were: malaoxon (*S*-1,2-bis(ethoxycarbonyl)ethyl) *O,O*-dimethyl phosphorothiolate), the diethyl homolog of malathion (*S*-(1,2-bis(ethoxycarbonyl)ethyl) *O,O*-diethyl phosphorodithioate), acethion (*S*-(ethoxycarbonyl)methyl *O,O*-diethyl phosphorodithioate), a compound of low mammalian toxicity described by O'Brien *et al.* (1958), and the dimethyl homolog of acethion (*S*-(ethoxycarbonyl)methyl *O,O*-dimethyl phosphorodithioate).

RESULTS.—The results of the studies with a series of insecticides are given in table 1. Most of the materials were of similar toxicity to larvae of both the R and S colonies. Of the organophosphorus compounds, only Co-Ral was appreciably less toxic to the R colony, and here the difference did not exceed a threefold factor. There was no difference in susceptibility to dimethoate, where detoxification by formation of a carboxylic acid derivative has been shown to occur in mammals (Dauterman *et al.* 1959). Phosdrin, which possesses a carbomethoxy grouping, was also identical in toxicity to both colonies. The R colony was resistant to both DDT and dieldrin. Here also, the difference in susceptibility did not exceed a threefold factor.

The results obtained when the compounds closely related to malathion were tested are given in table 2. Definite cross-resistance was obtained with malaoxon

Table 1.—Comparative susceptibility of larvae of malathion-resistant (R) and -susceptible (S) colonies of *Culex tarsalis* to a series of organophosphorus insecticides.[a]

Compound	Colony	Insecticide in Distilled Water (p.p.m.)										
		10	3	1	0.3	0.1	0.03	0.01	0.003	0.001	0.0003	0.0001
Malathion	R	100	67	20	0	0	0	0				
	S	100	100	100	100	100	33	0				
Demeton	R			100	0							
	S			100	0							
Dimethoate	R			100	0							
	S			100	0							
Dipterex	R			100	52	0						
	S			100	76	0						
DDVP	R				100	76	6	0				
	S				100	46	2	0				
Oxygen analog of ronnel	R				100	99	26	8	0			
	S				100	93	24	6	0			
Ronnel	R					100	94	28	0			
	S					100	100	34	0			
Phosdrin	R					100	0					
	S					100	0					
DOW ET-15	R					100	96	22	0			
	S					100	98	28	0			
Delnav	R					100	98	14	0			
	S					100	76	4	0			
Co-Ral	R					100	52	4	0			
	S					100	100	62	0			
Para-oxon	R					100	30	20	0			
	S					100	14	0	0			
Chlorthion	R						100	85	6	4	0	
	S						100	75	4	0	0	
Parathion	R							100	93	0		
	S							100	88	0		
EPN	R								100	68	10	
	S								100	55	0	
DDT	R				100	98	22	0	0	0		
	S				100	93	59	24	16	0		
Dieldrin	R					100	92	80	46	18	18	0
	S					100	100	100	96	32	18	16

[a] Per cent mortality in 24 hours.

Table 2.—Comparative susceptibility of larvae of malathion-resistant (R) and -susceptible (S) colonies of *Culex tarsalis* to malathion and closely related compounds.[a]

Compound	R Colony		S Colony		Ratio R to S	
	LC-50	LC-90	LC-50	LC-90	LC-50	LC-90
Malathion	2.4	4.0	0.040	0.045	60	89
Malaoxon	0.3	0.55	0.040	0.058	7.5	9.5
Diethyl homolog of malathion	0.45	0.675	0.062	0.115	7.2	5.9
Acethion	2.6	3.8	2.9	4.0	0.90	0.95
Dimethyl homolog of acethion	4.0	8.0	5.4	8.5	0.74	0.94

[a] P.p.m. of insecticide for indicated mortality in 24 hours.

and the diethyl homolog of malathion, but the degree of resistance was only 7-fold as compared with the 60-fold difference obtained with malathion. Acethion and its dimethyl homolog were of similar toxicity to both colonies. With both these compounds, the R colony was actually slightly more susceptible. The low toxicity of acethion and the lack of cross-resistance here were considered surprising in view of the close structural relationship of these compounds to malathion.

Three facts emerged from the bioassay studies: (1) there was almost no resistance to other organophosphorus insecticides, (2) resistance to chlorinated hydrocarbons was very low, and (3) possession of a single carbethoxy ester group did not confer resistance, decreased susceptibility occurring only when two such groupings were present.

MALATHION AND ACETHION DETOXIFICATION STUDIES.—Possible differences in the rate and/or nature of the detoxification of malathion and acethion were investigated, phosphorus-32-labeled samples of the compounds being used. Specifically, it was felt that increased ability of the resistant strain to detoxify malathion, presumably by hydrolysis at the carbethoxy ester linkages might be responsible for the resistance present. The use of acethion where the same type of hydrolysis is possible would show whether or not this method of detoxification occurred with an insecticide to which neither colony of mosquitoes was very susceptible.

Rate of Detoxification.—Both malathion and acethion were determined radiometrically to partition at least 98% into chloroform from water at neutral pH's. Their possible breakdown products, phosphoric and carboxylic acids, being ionized compounds, are not readily extractable from water at neutral pH's. Chloroform-water partitions were therefore used to separate the insecticides from their breakdown products.

Two hundred and fifty fourth-instar larvae were placed in 250 ml. of distilled water treated with sublethal concentrations of acethion or with concentrations of malathion sublethal to larvae of both colonies or lethal to larvae of the susceptible colony only. The same concentrations of insecticide in water with no larvae present were used as controls. At several times after treatment, duplicate 5 ml. samples of the test solution were removed and partitioned with equal volumes of chloroform to determine the rate of hydrolysis of the insecticide due to metabolism by the larvae. No attempts were made to characterize the radioactivity present in the larvae themselves, as only very small amounts were present at any particular time. It was assumed, therefore, that any absorbed insecticide was excreted almost as fast as it was hydrolyzed.

The results of these studies are summarized in table 3. Malathion was detoxified rather slowly by larvae of both colonies, but acethion was more rapidly destroyed. The more rapid hydrolysis of acethion by larvae of both colonies may be a satisfactory explanation of the much lower toxicity of this compound. No consistent pattern was obtained with malathion that could explain the large difference in susceptibility between the two colonies. The much slower rate of hydrolysis is sufficient to explain the higher toxicity of this compound to the S colony, but the fact that the R colony failed to hydrolyze the insecticide at an appreciably more rapid rate indicates that the 60-fold difference in susceptibility between the two colonies may be associated with factors other than the overall rate of hydrolytic metabolism.

Nature of Detoxification.—Through the use of ion-exchange chromatography, Seume & O'Brien (1960) have demonstrated that the major detoxication products of malathion in mice are carboxylic acid derivatives. Of the three possible carboxylic acids, the two monoesters, respectively *S*-(1-ethoxycarbonyl-2-carboxyethyl) *O,O*-dimethyl phosphorodithioate, and *S*-(1-carboxyethyl-2-ethoxycarbonyl) *O,O*-dimethyl phosphorodithioate, are extractable from water with chloroform at pH 3, while the dicarboxylic acid, *S*-1,2-dicarboxyethyl *O,O*-dimethyl phosphorodithioate, cannot be recovered in this manner (DuBreil 1960). The other possible hydrolytic metabolites of malathion, phosphoric and phosphorothioic acids, are stronger acids and cannot be extracted from water at acid strengths of less than pH 2. The technique of extracting water solutions of malathion metabolites at different pH's is satisfactory, therefore, for separating the two major groups of possible breakdown products, with the single exception of the dicarboxylic acid derivative

Table 3.—Rate of detoxification of P^{32}-labeled malathion and acethion by malathion-resistant (R) and susceptible (S) larvae of *Culex tarsalis*.[a]

Hours After Treatment	Acethion 2.5 p.p.m.			Malathion 0.047 p.p.m.			Malathion 1 p.p.m.[b]		
	R	S	C[c]	R	S	C[c]	R	S	C[c]
1	95	97	97	100	100	100	93	94	93
2	92	92	97	99	92	100	94	91	92
4	88	86	98	88	100	100	94	89	97
24	45	27	91	96	92	100	78	86	91
48	—	—	—	66	66	98	—	—	—

[a] Per cent radioactivity partitioning into chloroform.
[b] All susceptible larvae died before 6 hours.
[c] Control: no larvae.

Table 4.—Per cent of hydrolytic metabolites of malathion and acethion partitioning into chloroform at indicated pH's when solutions were exposed to malathion-resistant (R) and -susceptible (S) larvae of *Culex tarsalis* for 24 hours.

pH (HCl)	Malathion[a]		Acethion	
	R	S	R	S
7	11	14	3	0
6	10	23	9	3
5	41	25	3	3
4.5	43	36	13	11
4.0	50	47	66	43
3.5	61	56	87	89
3	58	52	90	93
2	55	57	95	94
1	68	71	93	93
0	67	58	93	94

[a] Partly lethal to susceptible larvae.

which would partition with the phosphatase derivatives when handled in this manner. However, Seume & O'Brien have shown that the dicarboxylic acid is only a minor fraction of the total metabolites from mice. Similar techniques have been applied to studies of the detoxification of dimethoate (Kaplanis *et al.* 1959) and acethion (O'Brien *et al.* 1958).

Groups of 250 larvae of each colony were exposed to sublethal concentrations of acethion and to a concentration of 0.1 p.p.m. malathion which was partly lethal to the susceptible colony, in 250 ml. of water. After 24 hours the larvae were filtered out, the pH of the solution readjusted to 7 with 0.1 N sodium hydroxide and hydrochloric acid, and the water partitioned with equal volumes of chloroform to remove any unmetabolized insecticide. The water was then acidified with hydrochloric acid and aliquots were partitioned with chloroform at various pH's.

The results of this experiment are summarized in table 4. With malathion 50% to 60% of the metabolites partitioned into chloroform from water at pH 3 with both colonies, and with acethion, 90%. The results provide evidence that carbethoxy ester hydrolysis is a major metabolic pathway with both resistant and susceptible mosquito larvae. The results also indicate that differences in the nature of the hydrolytic metabolism of malathion, insofar as the formation of carboxylic acid derivatives is concerned, are not important in the difference in susceptibility between the two colonies.

To substantiate the pH partitioning method, male white mice were treated orally with 500 mg./kg. of P^{32}-labeled malathion and the urine was collected for 24 hours after treatment. A portion of the urine was diluted with water, acidified with hydrochloric acid, and aliquots partitioned with chloroform as described with the mosquitoes. The results were almost identical to those obtained with the mosquitoes. At pH 3, 45% of the water soluble metabolites partitioned from water into chloroform and at pH 0, 62% of the metabolites partitioned into chloroform.

Another portion of the mouse urine containing malathion metabolites was chromatographed on a Dowex 1-X8, anion exchange resin, column (Plapp & Casida 1958) using the elution gradients described by Seume & O'Brien (1960). The elution position of a series of possible malathion metabolites was determined by chromatographing known samples of each compound on identical columns. The known metabolites studied included dimethyl phosphate, dimethyl phosphorothioate, dimethyl phosphorodithioate, and the mono- and dicarboxylic acid derivatives of malathion, respectively, *S*-(1-ethoxycarbonyl-2-carboxyethyl) *O,O*-dimethyl phosphorodithioate, and *S*-1,2-dicarboxyethyl *O,O*-dimethyl phosphorodithioate. When a sample of the labeled mouse urine was chromatographed on an identical column, it was found that 59% of the total radioactivity was eluted from the column in the same position as the mono- and dicarboxylic acid derivatives of malathion, a figure in good agreement with the results obtained by pH partitioning. At least five other hydrolytic metabolites were separated on this column, but no further attempt was made to characterize these other derivatives.

Ion exchange chromatography could not be used in characterizing the mosquito-produced metabolites of malathion as insufficient quantities were available, owing to the slow rate of malathion metabolism by mosquito larvae. However, with the less toxic and more rapidly metabolized acethion, sufficient quantities of metabolites were obtained from both R and S mosquitoes to permit identification by ion-exchange chromatography. Metabolites were chromatographed using the same elution gradients as for malathion. The results are given in table 5 and are in good agreement with the results obtained by pH partitioning; *i.e.*, by both methods of analysis 90% of the total derivatives behaved as carboxylic acids.

In another experiment, mice were treated orally with labeled acethion in peanut oil at 500 mg./kg. and the urine collected for 24 hours after treatment. The metabolites were characterized by paper chromatography using the acetonitrile:water, 85:15, system on untreated strips of Whatman No. 1 paper as described by Kaplanis *et al.* (1959). Known samples of three possible acethion metabolites, diethyl phosphate, diethyl phosphorothioate, and diethyl phosphorodithioate, were chromatographed with urine samples. Colorimetric determinations of the known compounds on developed chromatographs were made by the method of Hanes & Isherwood (1949). The R_f's of the mouse urine metabolites of acethion are listed in table 5. It was found that two metabolites with R_f values different from those of three known derivatives of acethion were the major compounds present. The compound at R_f 0.81 comprised more than 50% of the total metabolites and is probably the carboxylic acid derivative of acethion (*S*-carboxymethyl *O,O*-diethyl phosphorodithioate) (O'Brien *et al.* 1958). The other unknown metabolite (R_f

Table 5.—Metabolites of acethion as characterized by ion-exchange chromatography after exposure to larvae of malathion-resistant (R) and -susceptible (S) *Culex tarsalis*, and R_f's of mouse-urine metabolites as determined by paper chromatography.

Metabolite	Per Cent		R_f
	R	S	
Diethyl phosphoric acid	5	4	0.23
Diethyl phosphorothioic acid	2	3	0.46
Diethyl phosphorodithioic acid	1	3	0.65
Unknowns—probably carboxylic acids	92	90	0.57, 0.81
Acethion	—	—	1.00

0.57) is possibly the carboxylic acid derivative of the oxygen analog of acethion.

DISCUSSION.—The almost complete lack of cross-resistance to other organophosphorus insecticides in the mosquito colony resistant to malathion differs from the pattern usually found in phosphate-resistance studies. Studies with the house fly have indicated that resistance to one compound usually induces resistance to at least a number of related compounds (Brown 1960). Oppenoorth (1959) found that a strain of flies selected with malathion was highly resistant only to that compound and possessed a low cross-resistance to other dimethyl phosphates, similar to that present in parathion and diazinon-selected strains. Busvine (1959) found that a malathion-selected strain was highly resistant to a number of other dimethyl phosphates, but that resistance to dimefox, DDVP, and a series of diethyl phosphates was low.

It is known that with house flies, resistance to organophosphorus compounds, usually results in much higher levels of resistance to chlorinated hydrocarbon insecticides. March (1959) has shown that colonies of flies selected with parathion, Chlorthion, and malathion are nearly immune to both DDT and dieldrin. Resistance to both these compounds is much lower than the resistance to malathion in the R colony, and this difference is probably the most outstanding variation from the pattern usually found in phosphate resistance studies. Resistance to DDT was present in the R colony at the time it was originally collected and it is not known if it is dependent on the resistance to malathion or is due to other factors.

The resistance to malaoxon and the diethyl homolog of malathion and the lack of cross-resistance to either acethion or its dimethyl homolog shows that the only grouping common in compounds to which resistance occurs is the possession of the 1,2-bis(ethoxycarbonyl) ethyl grouping. However, this does not seem to be the only factor, since the resistance to both malaoxon and the diethyl homolog of malathion is much less than to malathion itself. The cross-resistance to malaoxon agrees with Oppenoorth's observation (1959) that resistance is not due to a failure in the mechanism which converts malathion to malaoxon.

A factor to be considered in evaluating the results obtained is the method of testing employed. Here, rather than topical application or brief exposure to a spray, the insects were exposed constantly to the insecticide for the entire period of the bioassay. In more recent tests, adults of the two colonies were exposed to residues of several of the insecticides tested against larvae. The results were in general agreement with those obtained previously. Adults of the R colony were about 100 times resistant to malathion and both R and S adults were equally susceptible to parathion.

The data failed to show any substantial differences in either the rate or the nature of malathion detoxification between the two strains. The evidence presented for the detoxification of the insecticide by carbethoxy ester hydrolysis is admittedly incomplete. However, it was not possible to obtain enough hydrolytic metabolites to perform analyses by other methods. Nevertheless, the close agreements with the results obtained from malathion metabolism in mice and the results obtained with acethion are strong evidence that the detoxification of the insecticide proceeds in the same manner in both colonies. This also implies that detoxification by carbethoxy ester hydrolysis is a major defense mechanism with both colonies, but not the critical defense factor in the case of malathion resistance. The results provide additional evidence that detoxification of insecticides by carbethoxy ester hydrolysis is a phenomenon occurring in insects as well as warm-blooded animals.

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A Trap to Quantitatively Recover Dead and Abnormal Honey Bees from the Hive[1]

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ABSTRACT

A device is described which efficiently traps dead and abnormal honey bees (*Apis mellifera* L.) from the hive under field conditions. The device is designed to utilize normal housecleaning activities of bees to automatically collect dead and abnormal bees in glass jars which may be removed at desired intervals for tabulation of data. Almost 100% of queens and drones are recovered while approximately 80% to 90% of worker bees are recovered. Data are given which establish the normal range of mortality in populous colonies.

New methods for intrahive testing of insecticide toxicity to honey bees and evaluation of adult bee diseases are made possible by the use of the trap.

In several phases of apicultural research it is essential that honey bee mortality within the hive be evaluated accurately. The "dead bee trap" herein described was originally designed for recovering dead queens in a series of experiments on forced supersedure (Gary 1959). During these experiments it was discovered that dead worker bees and drones were also collected efficiently. Consequently, the queen supersedure experiments were expanded to include a simultaneous study of normal drone and worker mortality as well as to determine the collection efficiency of the trap.

The dead bee trap is useful in obtaining an index of bee losses caused by insecticide applications. Preliminary evidence gathered by Morse (1959) during a survey of bee losses from Sevin® (1-naphthyl methylcarbamate) indicates that many foraging bees are able to return to the hive before they die. In some cases foraging bees may gather pollen which is contaminated with an insecticide. Such pollen is consumed within the hive by nurse bees, resulting in abnormal mortalities which may persist several days after insecticide application. Intrahive mortalities are of further significance in toxicity studies in which individually-treated bees are introduced and recovered from normal colonies. Studies concerning adult bee diseases and longevity determinations may also require an evaluation of intrahive mortality.

Several attempts have been made to construct an apparatus to collect dead bees at the hive. McIndoo (1926), Rennie (1927), Herman & Brittain (1933), Johansen *et al.* (1957), and Anderson & Atkins (1958a, b) have reported the construction of various devices to collect dead bees at the hive. All of the devices suffered certain shortcomings in that they were either (1) structurally incapable of efficient collection and retention of both crawlers and dead bees (2) not weather resistant or (3) not designed to accumulate bodies in removable containers for easy sample collecting. In no cases were control worker bodies introduced into the traps in order to determine the daily loss of sample due to housecleaning bee and scavenger activities. Without controls it is impossible to correct the observed body recovery so that an accurate estimate of the true mortality in each hive can be made.

HOUSECLEANING BEHAVIOR.—A knowledge of housecleaning behavior is necessary before the design and function of the trap can be fully appreciated. Housecleaning behavior concerns the removal of debris, dead and abnormal bees, and foreign insects and materials from the hive. Objects light in weight are carried by the bees high into the air and presumably dropped far from the hive. However, with heavier loads such as bee bodies, the housecleaner bees usually fall to the ground beneath the entrance. Thereafter the bees alternately drag the bodies and attempt to fly until the bodies are finally abandoned. Those bodies abandoned within several feet of the colony entrance will often be removed later by other bees, especially if there is an open flat surface where the bodies are exposed. Eventually many of the remaining bodies are consumed by scavengers, either insects or small mammals. Wasps were the most active scavengers observed. It is therefore not surprising that the number of dead bees found on the ground around a colony may bear little relation to the mortality within the hive except under severe conditions of excessive mortality.

A study of normal housecleaning behavior indicates that any device designed to collect bodies must (1) create a physical barrier which completely prevents escape by crawling, and (2) restrict flight by housecleaners, at the same time providing for normal forage bee flight.

TRAP DESIGN AND FUNCTION.—The trap is rectangular in shape and made of 1/8-inch mesh hardware cloth (fig. 1). This material has the necessary qualities of weather resistance, low cost, and ease of fabrication. The trap width is determined by the width of the hive used. The upper edges of the three walls are folded inward to provide a 1 1/2-inch rim. To the underside of the rim is stapled a 1 1/2-inch wide strip of 300A Mylar® polyester film[2] or 20-gauge celluloid sheet which overlaps at the outer two corners to provide a smooth continuous band. On the fourth side immediately above the hive entrance this band is made continuous by a glass strip 1×1/8×16 3/8 inches that is held firmly in position by tension from an overlapping metal clip on each side. The metal clips are soldered onto the rim top. This structure is necessary in order to allow sufficient flexibility of the sidewalls nearest the hive entrance to properly fit hives that have slight variations in widths under field conditions. The underside of the smooth continuous band is covered with a thick layer of petroleum jelly (petrolatum) which effectively prevents the escape of crawling bees. Glass or other smooth-surfaced materials in addition to those previously mentioned could also be used to support the grease layer.

In order to restrict the flight of housecleaner bees a partial lid 8 3/4×16 3/8 inches made of 1/8-inch mesh hardware cloth is placed on the trap top. It rests on the rims in a position which leaves a 2-inch open slot along the lid edge over the hive entrance and a 1-inch open slot along the lid edge furthermost from the colony. The

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[2] Distributed by Brownell Distributors, 306 Canal Street New York, N. Y.

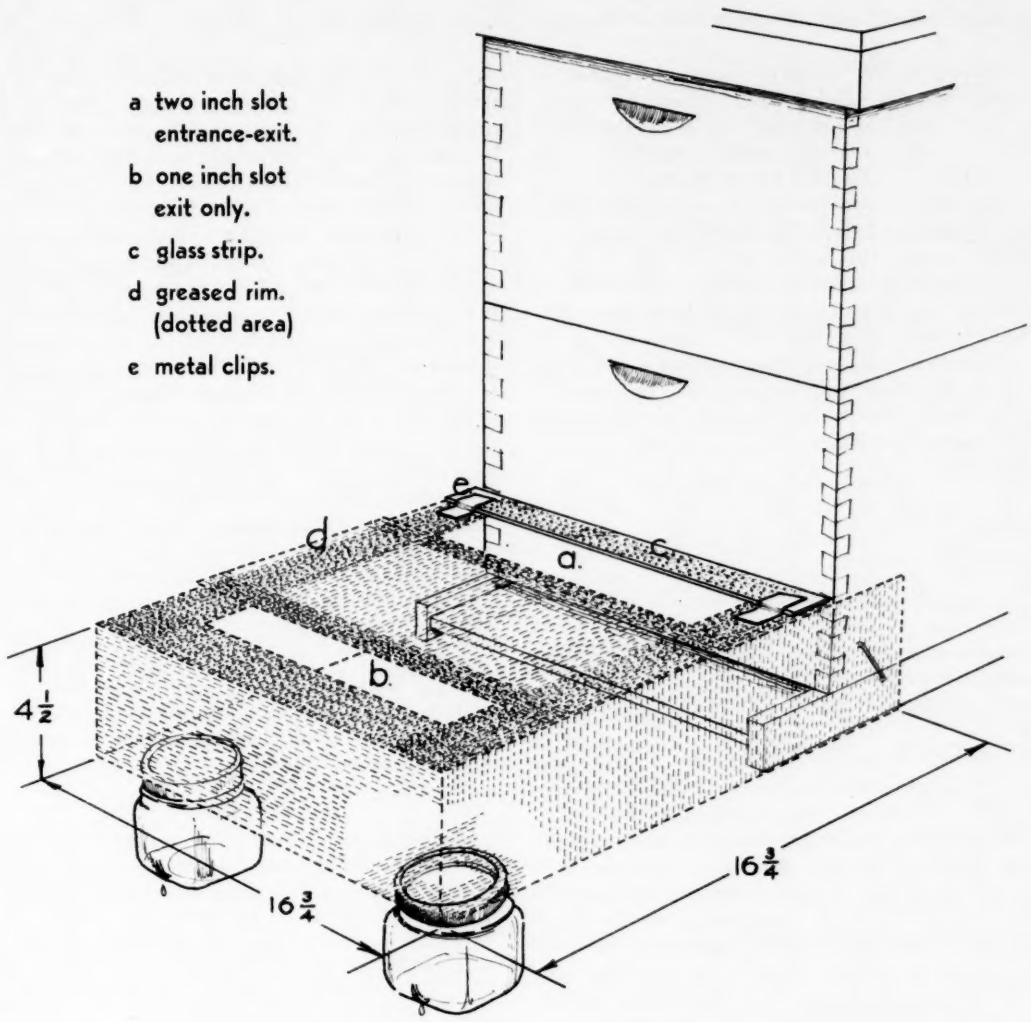

FIG. 1.—Dead bee trap attached to hive.

undersides of the lid edges adjacent to both slot openings are also covered with a 1 1/2-inch strip of a smooth-surfaced material which is greased with petroleum jelly.

Since the grease barrier prevents escape by crawling, bees can only escape by flying through the open slots. Normal outgoing forage bees are able to fly easily through either opening after a brief training period. Crippled bees and housecleaner bees heavily ladened with bee bodies are awkward in flight. They collide with the ceiling or sidewalls and fall to the floor; some do escape, however. Returning forager bees use the wide 2-inch slot almost exclusively. This wide opening eliminates a potential bee traffic congestion problem over the entrance and also provides an easily accessible entrance for poisoned bees that are able to return from the field. The outer 1-inch slot must be narrow since most of the housecleaners' attempts to escape occur in the outer three-fourths of the trap.

Two 1-inch square holes are made in the floor about 1 1/2-inches inward from the outer trap corners. Lids that fit wide-mouth screw-top pint jars are soldered to the underside of the floor around the openings. Housecleaners persistently crawl about the trap interior until they finally tumble through the holes into the jars either by accident or in search of an escape opening. Almost invariably they must abandon the bodies in the jars before they can crawl up the smooth inward-slanting glass sidewalls to escape. Crippled bees are usually unable to escape from the jars; if successful they repeatedly fall into the jars and eventually remain there. The trap structure therefore utilizes bee behavior to attain the automatic collection of dead and abnormal bees in containers for easy removal.

The traps are easily installed by telescoping 4 inches of the trap floor under the entrance and stapling the flexible sides to the hive walls. The open slit between the hive bottomboard and the trap floor should be blocked with a small stick. Hives must be supported on a simple hive stand. Immediately after installation some bees may return from foraging and cluster under the trap floor. If this behavior persists the trap floor level should be lowered or a barrier may be placed from the outer floor edges

to the ground to discourage attempts to enter from the underside.

After initiating routine mortality counts the petroleum jelly coating must be carefully maintained. Otherwise, "escape trails" may develop wherever voids develop in the coating. Jars should be cleaned occasionally to avoid the accumulation of a dirt film which affords traction to the glass sidewalls. It is desirable to grind a narrow slit in the bottom of each jar for the drainage of rain water.

The daily recovery percentage for each hive is determined by introducing a known number (20 to 50) of marked worker bee bodies to the colony early each morning through the inner cover hole in the top or well inside the hive on the bottomboard. These bodies are in effect controls to insure proper trap performance. The percentage recovery of the control bodies at the termination of bee activity each day enables an accurate estimate of the corrected mortality per colony:

$$\frac{1}{\%\text{ recovered controls}} \times \text{observed body recovery} = \text{corrected mortality}$$

Wide variations occur among colonies each day and within colonies on different days. Workers used for controls should approximate the weight of the bodies to be collected.

EXPERIMENTAL RESULTS.—Experiment 1 was performed to determine normal bee mortality and trap collection efficiency. An apiary near Ithaca, New York, consisting of 26 colonies, each occupying three to five standard Langstroth supers, was used. Vigorous bee flight prevailed during a heavy honey flow throughout the observation period whenever weather permitted. Four traps were allowed to remain on their respective hives for the duration of the period while the other traps were placed on different colonies midway during the period. The placement of traps was entirely dictated by the simultaneous collection of data on virgin queen mortality from mature queen cells introduced into queen-right colonies.

Early each morning on 8 different days 20 marked worker bee bodies, each weighing a mean of approximately 70 to 80 mg., were placed inside each colony on the bottomboard. Each evening at dusk after bee activity had ceased, the jars were removed and the recovery of marked and unmarked workers was tabulated (table 1).

All workers that were able to move any appendage were classified as living workers. Approximately 2% of the worker bodies remained on the trap floor at the termination of bee activity each day, thus demonstrating the efficient manner in which bee samples were collected in the sample containers. The average of the daily means for the per cent recovery of controls (table 1) is 84.6%. This figure represents the approximate efficiency with which the traps collected dead worker bee bodies. There is almost 100% recovery of drone and queen bodies due to their heavier weights (Gary 1959).

DISCUSSION.—Throughout these tests there was no evidence to indicate abnormal colony behavior owing to the presence of the traps. Neither did the housecleaner bees demonstrate any increase in ability to escape. Apparently the device may be used for long-term experiments without sacrificing either colony productiveness or data reliability. Adequate sample collections for disease studies, residue analyses, and for quantitative mortality data are thus assured with a minimum expenditure of labor and time.

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Table 1.—Trap efficiency and normal mortalities of workers and drones within the hive. Ithaca, New York. 1958.

		Aug. 22	Aug. 23	Aug. 24[a]	Aug. 26	Aug. 27	Aug. 28	Aug. 29	Aug. 30
Recovery of controls (%)	Mean	96	89	98	80	85	83	83	63
	Range	90–100	65–100	85–100	40–100	47–100	45–100	45–100	5–100
Dead drones	Mean	5	6	7	6	12	7	15	9
	Range	0– 17	0– 22	0– 35	0– 28	0– 48	0– 48	1– 90	1– 56
Dead workers[b] (corrected)	Mean	71	98	128	63	80	110	98	105
	Range	13–161	20–180	47–254	14–118	31–168	44–189	36–171	38–247
Living workers	Mean	5	5	8	9	7	2	4	0[c]
	Range	0– 21	0– 18	1– 24	0– 21	1– 15	0– 5	0– 12	0[c]
Temperature F.	Mean[d]	65	59	61	57	59	62	65	70
	Range	54– 75	45– 72	52– 69	44– 70	42– 75	48– 77	48– 82	57– 83
No. of colonies		15	12	15	15	15	15	14	13
Hours active flight		6	8	1	4	7	8	8	8

[a] Samples for August 24 collected 10:00 a.m., August 25.
[b] Corrected dead workers determined by formula in text.
[c] Samples held overnight before tabulation of data; living workers died.
[d] 24-Hour mean.

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Loss of Activity of Chlorinated Hydrocarbon Insecticides in Soil as Measured Against the Eye Gnat, *Hippelates collusor*[1]

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ABSTRACT

The long term effectiveness of DDT, endrin, lindane, dieldrin, aldrin and Shell Development Company Compound SD-4402 (1,3,4,5,6,7,8,8-octachloro-3*a*-4,7,7*a*-tetrahydro-4,7-methanophthalan) in soil was studied under controlled conditions against the eye gnat, *Hippelates collusor* (Townsend). Several lots of Coachella fine sand containing 0.15% organic matter dry weight basis were treated with sprays of each toxicant and stored in gallon mayonnaise jars in a glasshouse, maintained at 88 ± 2° F. The moisture content of the soil was maintained at 11% to 12% of the dry weight of the soil. The dosage of each material was adjusted to the initial LD_{90} level of activity. The activity of the toxicants was determined by bioassaying lots of the treated soil against the gnat at 3-month intervals.

No loss of activity was detected for DDT, endrin and lindane, 9 months after storage. Dieldrin also did not show loss of activity 12 months after start of the storage period under the test conditions. Aldrin and compound SD-4402 manifested appreciable loss of activity 3 months after start of the storage period. After the 3-month storage period 53% of aldrin and 40% of compound SD-4402 were not accounted for. The magnitude of loss of aldrin further increased on storage and was determined to be 77% after a period of 9 months of storage and 70% after 12 months of storage. The loss of activity of compound SD-4402 increased rapidly from after the 3-month to the 6-month interval of storage and was determined to be 72% after a period of 6 months of storage. No further increase in loss of this material and of aldrin could be detected with the bioassay method used in these studies.

Hippelates eye gnats predominantly breed in cultivated loose soils containing adequate amounts of moisture and organic matter. Friable soils containing adequate moisture could breed a large number of eye gnats over prolonged periods of time without being subjected to disking or other form of disturbance. This type of land breeds gnats constantly but at a lower level, while cultivated grounds result in heavy gnat production only when preceded by irrigation and disking under of sufficient quantities of cover crop or weeds.

One way of abating pestiferous eye gnats is to incorporate residual insecticides into the soil. This approach has been employed to some extent both experimentally and as large scale soil larvicidal treatments. The initial effectiveness of various chlorinated hydrocarbon insecticides against *H. collusor* (Townsend) was investigated in the Coachella Valley of southern California and as a result of these studies aldrin was found to be the most effective one (Tinkham 1952, 1953). Aldrin, lindane and chlordane were found to have initial effectiveness against *H. pusio* Loew (Dow & Willis 1959). The long term effectiveness of several chlorinated hydrocarbon insecticides against eye gnats was studied in the Coachella Valley (Mulla *et al.* 1960) during 1956–57. Aldrin, dieldrin and heptachlor at economical or higher dosages when applied as sprays or granules gave poor intial control of *H. collusor*. These materials 7 to 9 months after application did not result in economical control of this gnat. Concurrent with these field tests the initial effectiveness of chlorinated hydrocarbon insecticides against *H. collusor* was determined under controlled laboratory conditions (Mulla 1960a). Compound SD-4402 (1,3,4,5,6,7,8,8-octachloro-3*a*,4,7,7*a*-tetrahydro-4,7-methanophthalan), endrin, aldrin, DDT, dieldrin and lindane were found to be the most effective ones. The loss of the first four insecticides of this group from soil surface was studied under field conditions (Mulla 1960b) and DDT was found to be the most stable material.

The long term effectiveness of a material in soil against insects will depend on many factors some of which are the stability of the toxicant, conversion to other stable or more effective substances and the biology of the pest to be controlled. Long lasting control of soil insect pests having long life cycles, low population density, low reproductive potential and low capability to reinfest controlled areas may not be an indication of the residual activity of an insecticide employed. *Hippelates collusor*, the most predominant eye gnat in the southwest, does not have any of these biological attributes. Duration of its life cycle is short (10 to 20 days), maintains high population density in the infested areas, has a high reproductive potential and the females are mobile and capable of infesting other areas in a short time (Mulla & March 1959). Therefore, long lasting control of *H. collusor* with soil larvicidal treatments can be only obtained by employing effective long lasting insecticides. Study of the residual effectiveness of insecticides against eye gnats in the field is a tedious task and at times may not be too reliable. However, with the availability of the laboratory colonies, it is now possible to

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[2] The assistance of D. G. McHaffey during the course of these studies is acknowledged.

study the long term effectiveness of various insecticides in soil against the pests thus selecting more stable materials for field evaluation with a minimum effort and expense. The longevity or long term effectiveness of DDT, endrin, lindane, dieldrin, aldrin and compound SD-4402 as soil treatments against *H. collusor* under controlled conditions has been studied and the results are presented here.

EXPERIMENTAL PROCEDURE.—Coachella fine sand was air-dried and sifted through a 40-mesh sieve to remove coarser debris. The organic matter content of this soil was determined to be 0.15% dry weight basis. Four 1-gallon (11.5 lbs./gallon) quantities of this dry soil were treated with dilute sprays of each material prepared from the emulsifiable concentrate formulation of the toxicant. A 36-square-inch area of each soil lot was sprayed with 2.32 ml. of the dilute spray by means of a DeVilbiss atomizer. This corresponded to applying the spray at the rate of 100 gallons per acre and yielding the indicated dosages of toxicants per acre of soil 6 inches deep.

After the spray had dried the soil was mixed thoroughly and placed in gallon mayonnaise jars. Initially 700 ml. of water was added to the soil in each jar and the jars were covered with muslin cloth. The soil was stored in a glasshouse maintained at 88 ± 2° F. At 3-month intervals additional 350 to 400 ml. of water was added to each of the remaining jars to maintain the moisture level at approximately 11% to 12% dry weight basis.

The treated soil was tested at 3-month intervals for the longevity of the toxicants against the eye gnat, *H. collusor*. Each soil lot was spread out on benches and air dried. The clods were broken and the soil mixed thoroughly. Organic matter was added to each gallon of soil in the following quantities: alfalfa leaf meal 56 gm., Brewer's yeast 28 gm., and whole wheat flour 28 gm. The organic matter was uniformly mixed with the treated soil. After mixing, the soil was placed in 10 narrow-mouthed pint jars in equal amounts, with each treatment thus being replicated 10 times. The soil in each pint jar was moistened with 140 ml. water initially. Subsequently, additional water was added as needed. Each jar was seeded with 200 eye gnat eggs 1 to 3 days after addition of the water. The eye gnat colony was started in the fall of 1957 from wild populations collected in the Coachella Valley. The colony was in its 25th generation at the start of these tests and in its 35th generation at the conclusion of the experiments. After addition of the eggs the jars were covered with 50-mesh strainer cloth disks and the eye gnat emergence was recorded for each jar.

Only ½-gallon quantities of soil were treated for obtaining the initial activity of the toxicants. Also, in the control treatments the quantity of soil used per test was one-half gallon. The quantity of organic matter added was also cut in half and the soil was placed in five narrow-mouthed pint jars, thus resulting in five replicates for each treatment. The fresh and control treatments were handled in the same manner as described before.

The jars of each treatment were arranged in randomized block design on a glasshouse bench where the temperature was maintained at 88 ± 2° F. throughout the development and emergence of the eye gnats. Emerging gnats were anesthetized with carbon dioxide and counted at 2- to 3-day intervals.

The per cent reduction in eye gnat emergence for each treatment was obtained by reference to the control treatment. The per cent loss was determined by reading off the amount of the toxicant corresponding to a reduction in eye gnat emergence from dosage mortality lines established for each material against the test insect (Mulla 1960a). The degree of emergence from freshly treated soil with each toxicant was also used as a guide in arriving at the loss of the activity of the materials tested.

RESULTS AND DISCUSSION.—The biological activity of DDT, endrin and lindane in soil on storage was measured 3, 6 and 9 months after start of the storage period. Owing to no apparent loss of these materials after 9 months of storage, their biological activity was not measured 12 months after start of the storage period.

DDT was applied to the soil at the rate of 26 pounds actual material per acre. Endrin and lindane were applied at the rates of 4.8 and 32 pounds actual material per acre, respectively. These rates of application corresponded to the initial LD_{90} levels of these materials in soil against *H. collusor* (Mulla 1960a).

The aged and fresh DDT treatments at all intervals resulted in approximately 90% or more reduction of the emerging gnats (table 1). Endrin treatments also resulted in a similar reduction of eye gnat emergence from the treated soil. Lindane treatment, after 3 months of storage gave poor reduction (78%) of the emerging gnats but subsequent ages of lindane treatments gave excellent reduction of the emerging gnats. This suggests that the poor performance of the 3-month-old lindane treatment could be due to experimental variation.

Dieldrin, aldrin, and compound SD-4402 were applied at the rates of 30, 18, and 1.5 pounds actual toxicant per acre. These rates approximated the initial LD_{90} levels for these materials against *H. collusor* (Mulla 1960a).

Table 1.—Longevity of DDT, endrin and lindane in soil as tested against the eye gnat, *Hippelates collusor*.[a]

Material and Formulation	Toxicant Acre (lb.)	Average Emergence of Gnats/Jar[b] and Reduction (%) After Months Shown											
		0		3		0		6		0		9	
		G/J	%R	G/J	%R	G/J	%R	G/J	%R	G/J	%R	G/J	%R
DDT EC 2	26	8	92	11	89	2	99	1	99	1	99	1	99
Endrin EC 1.6	4.8	11	89	12	88	3	98	15	89	1	99	7	94
Lindane EC 1	32	5	95	22	78	3	98	6	96	1	99	8	93
Control	—	100	—	—	—	143	—	—	—	112	—	—	—

[a] No appreciable loss was detected in any of the treatments up to 9 months of age.

[b] G, gnats; J, jar; %R, per cent reduction. All the aged treatments were replicated 10 times and the fresh and control treatments were replicated five times in a completely randomized block design.

Table 2.—Longevity of dieldrin, aldrin and compound SD-4402 in soil as tested against *Hippelates collusor*.

Material and Formulation	Toxicant Acre (lb.)	Average Emergence of Gnats[a] and Reduction (%) After Months Shown															
		0		3		0		6		0		9		0		12	
		G/J	%R	G/J	%R	G/J	%R	G/J	%R	G/J	%R	G/J	%R	G/J	%R	G/J	%R
Dieldrin EC 1.5	30	5	96	1	100	0	100	0	100	3	98	5	96	0	100	2	98
Aldrin EC 2	18	6	95	45	65	14	88	52	55	10	92	82	30	0	100	63	42
SD-4402 EC 1.25	1.5	29	78	70	45	1	99	95	17	26	78	101	14	21	80	77	28
Control	—	128	—	—	—	115	—	—	—	117	—	—	—	104	—	—	—

		Average loss after months shown (%)[b]			
		3	6	9	12
Dieldrin EC 1.5	30	0	0	0	0
Aldrin EC 2	18	53	60	77	70
SD-4402 EC 1.25	1.5	40	72	74	66

[a] G, gnats; J, jar; %R, per cent reduction. All the aged treatments of the insecticides were replicated 10 times in a completely randomized block design and the fresh and control treatments were replicated five times in the same manner.
[b] Per cent loss was calculated from standard dosage mortality lines of each material.

No loss of biological activity was detected for dieldrin (table 2) over a period of 1 year's storage. Approximately one-half of the activity of aldrin was lost 3 months after start of the storage period. The loss of activity of compound SD-4402 was 40% after the first interval of the storage period, but reached a peak after 6 months of storage. A loss of activity of 66% to 74% was ascertained for this material at the 6- to 12-month intervals (table 2).

The loss of activity of aldrin increased from 53%(after 3-month interval) to 60% after the 6-month interval. Its loss of activity reached a peak after 9 months of storage. No further loss in activity was detected beyond 9 months of storage. The lower magnitudes of loss of aldrin and compound SD-4402 obtained after 12 months of storage are within the limits of experimental variation.

The trend of the loss of activity of aldrin in soil in the study may be explained in terms of the conversion of this material to its epoxide (dieldrin) in soil (Bollen *et al.* 1958, Gannon & Bigger 1958). The range of activity of dieldrin in soil against *H. collusor* was found to be approximately one-half of that of aldrin (Mulla 1960a, 1960b). The reduction in emergence of the gnats produced by the aldrin treatments at the 3- and 6-month intervals correspond to what might be produced by an equivalent rate of application of dieldrin. The decrease in per cent reduction at subsequent intervals (9 and 12 months) could be attributed to loss of activity of dieldrin, when present in lower quantities.

The loss of activity of chlorinated hydrocarbon insecticides cannot be measured accurately by the bioassay method used in this study when these materials are present in small quantities. Loss of activities up to 70% or 80% may be measured by this procedure. On further loss the amounts of toxicants present would be too small and these small quantities would cause no appreciable reduction in the eye gnat emergence. This is owing to the low susceptibility of *H. collusor* to these materials excluding compound SD-4402. Notwithstanding this limitation the testing procedure has yielded good information in determining the longevity of chlorinated hydrocarbon insecticides against the most predominant species of eye gnats in the southwest.

It is expected that the rate of loss of toxicants under more severe conditions in the field would be greater than that observed in the laboratory. However, it is expected that the relative position of the long term effectiveness of the various toxicants would more or less pattern after laboratory findings. Laboratory studies show that aldrin, even though applied at a very high dosage (18 lbs./A) would not yield long term control of *H. collusor*. On the other hand, compound SD-4402 although losing its activity rapidly in the soil may give prolonged practical control of this species when the material is applied at relatively higher dosages.

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Hexachlorocyclopentadiene, A Promising New Insecticide for the Control of the Root Form of the Grape Phylloxera[1]

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ABSTRACT

In field tests on the control of grape phylloxera, *Phylloxera vitifoliae* (Fitch), on the roots of Concord grape, observations indicated that hexachlorocyclopentadiene was effective against the insect. The fumigant appeared to be effective at dosages of 150 to 200 pounds per acre. Applications were made with a hand injector and as a drench to the surface of the soil. No injury was evident on the roots of Concord grape at a dosage of 300 pounds per acre.

The grape phylloxera, *Phylloxera vitifoliae* (Fitch), is a native American insect that has probably always been present on wild grape vines. This insect appears in two distinct forms on the vine. The leaf form produces spherical galls on the underside of the leaves, and the root form lives on the roots and causes gall-like swellings or enlargements. The leaf form is the noticeable one and is rather common on our wild grape vines. The root form is not readily observed but is the one that may cause the greatest damage to the vine.

Although some varieties of American grapes are resistant to the root form, the variety Concord is subject to attack and it is not uncommon to find the roots of this variety injured by the insect. The leaves of Concord are seldom if ever injured.

Since the grape phylloxera occurs on the roots of Concord vines in many commerical vineyards, investigations were started in 1955 to study the control of this insect.

In 1955 preliminary tests were conducted with the following compounds: dibromohexachlorocyclopentene, dibromotrichloroethane, dibromotetrachloroethane, n-bromobenzamide, n-bromo-m-chlorobenzamide, pentachloropentadienoic acid, hexachlorocyclopentene-2-1, hexachlorocyclopentene-3-1, pentachloro-2-cyclopentenone, octachloromethylene cyclopentene, bis(pentachlorocyclopentadienyl), perchlorofulvalene, hexachlorocyclopentadiene polymer, compound C_{10} CL_{100}-(H-1), hexachlorocyclopentadiene, and polymer chloroethylenes.

The results of the tests indicated that the compound, hexachlorocyclopentadiene was toxic to the insect and was worthy of further consideration. This report is a summary of the results for 1956–58 with hexachlorocyclopentadiene against the root form of the grape phylloxera.

METHODS AND MATERIALS.—The plants were of the variety Concord and seedling grape vines. Some of the vines were planted in a single row and others were planted in cardboard cylinders (25 inches high and 24 inches in diameter). Before the vines were planted each container was sunk into the soil sufficiently so that the top was flush with the surface. A single vine was planted in each container.

During the summer the vines were infested with grape phylloxera by placing leaf galls, containing eggs and crawlers, on the roots of each plant. The young crawlers readily colonized on the roots and gall formations (fig. 1) were found 12 days after the plants were inoculated.

FIG. 1.—Gall formations on the roots of a Concord grape vine.

The effectiveness of the various treatments was determined by carefully removing the plants from the soil and counting the number of living insects on a certain number of galls. In some tests the effectiveness of a given treatment was determined by counting the number of galls on the roots of the plants. Only gall formations were considered that had living insects clinging to the roots. Untreated vines from adjacent plots were examined in a similar way. The rates of application given per acre refer to overall coverage. All the tests were conducted on grape vines growing in Conotton gravelly loam soil.

In the tests where the fumigant was injected into the soil, the required amount of toxicant for each injection was diluted with Stoddard solvent to make 3 ml. of solution per injection. The fumigant was applied with a hand-operated soil injector and the emulsifiable mixture was applied with a sprinkling can.

The fumigant, hexachlorocyclopentadiene,[4] is a light yellow liquid having a pungent odor.

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[3] G.L.F., Research and Development, Ithaca, N. Y.

[4] Manufactured by Hooker Electrochemical Company, Niagara Falls, N. Y.

Table 1.—Results of field experiments for control of grape phylloxera with hexachlorocyclopentadiene. 1957.

ACTUAL TOXICANT PER ACRE (LB.)	NO. GALLS EXAMINED	AVERAGE NO. OF INSECTS PER GALL
	Series 1[a]	
25	25	8.2
50	25	8.9
100	73	2.1
150	82	1.0
200	68	0.9
	Series 2[b]	
25	75	6.3
50	77	8.0
100	75	1.4
150	12	1.5
200	51	0.7
	Series 3[c]	
25	102	5.7
50	25	2.4
100	75	2.5
150	55	2.6
200	25	0.0
Check	237	7.6

[a] Applied 3 inches deep on 12- ×12-inch grid.
[b] Applied 6 inches deep on 12- ×12-inch grid.
[c] Applied 12 inches deep on 12- ×12-inch grid.

Physical data:

Formula	C_5CL_6
Molecular weight	272.79
Freezing point	9.6° C.
Boiling point	239° C. at 760 mm.
Refractive index N25/D	1.5625
Specific gravity 15.5°/15.5° C.	1.717
Pounds per gallon at 15.5° C.	14.30
Flash point (open cup)	None

TESTS IN 1956.—During the season a number of plants were treated with hexachlorocyclopentadiene at the rate of 95 and 190 pounds per acre. The material was injected into the soil at a depth of 6 inches on a 10-×10-inch grid. After an exposure period of 6 weeks the vines were carefully removed from the soil and a record made of the number of living insects on each gall. At 190 pounds per acre the fumigant was quite toxic to the insect. The material did not give a high degree of control at 95 pounds per acre.

TESTS IN 1957.—Grape vines infested with the insects were moved into the greenhouse in January. The vines were planted in metal containers (9 3/4 inches in diameter and 12 inches high). After the plants had resumed growth they were treated with the fumigant. Each treatment was replicated three times and a replicate consisted of a single vine. The chemical was injected into the soil at a depth of 6 inches and at two points, 3 inches from opposite sides of the plant. Hexachlorocyclopentadiene was used at the rate of 50, 100, 150 and 200 pounds per acre. Under greenhouse conditions all the treatments gave satisfactory results. The insect population was rather low on the plants and this fact may have had some influence on the degree of control.

In the field, tests were carried out with hexachlorocyclopentadiene to obtain information on the dosage and the depth of injection. The vines were 2 years old. Each plant was growing in a cardboard container. A list of the treatments is given in table 1. The treatments were replicated four times and a replicate consisted of a single vine. The fumigant was injected into the soil at two points 6 inches from either side of the plant. The vines were removed from the soil 6 weeks after the treatments were applied. The results are given in table 1. As may be observed the material was relatively ineffective at the 25- and 50-pound rates. At the 100-, 150- and 200-pound rates the insecticide greatly reduced the insect population.

Table 2.—Results of field tests for control of grape phylloxera with hexachlorocyclopentadiene.

ACTUAL TOXICANT PER ACRE (LB.)	AVERAGE NO. GALLS PER PLANT WITH LIVING INSECTS	
	1957	1958
	Series 1[a]	
100	6.0	42.0
150		8.3
200	0.7	3.2
Check	48.0	47.3
	Series 2[b]	
50	12.7	
100	3.6	41.1
150	4.0	54.5
200	1.0	19.3
Check	48.0	47.3
	Series 3[c]	
100		4.5
150		0.0
200		1.9
300		1.2
Check		47.3
	Series 4[c]	
200		0.0
300		0.0
Check		29.3

[a] Applied 3 inches deep on 12- ×12-inch grid.
[b] Applied 6 inches deep on 12- ×12-inch grid.
[c] Emulsifiable concentrate (95%) mixed with water and applied to the surface of the soil with a sprinkler using 2275 gallons of water per acre. Water seal 2275 gallons per acre.

Another series of experiments was performed to obtain information on the dispersion of the material from the point of injection. The vines were confined in cardboard containers. Hexachlorocyclopentadiene was used at the rate of 200 pounds per acre in all the treatments. The treatments were as follows:

(A) injections 3 inches deep and 8 to 12 inches from the plant,
(B) injections 6 inches deep and 10 to 12 inches from the plant,
(C) injections 12 inches deep and 10 to 12 inches from the plant,
(D) injections 24 inches deep and 12 inches from the plant,
(E) two injection points 24 inches deep and 6 inches from each side of the plant.

Six weeks after the treatments were applied the soil was carefully removed from the roots and a record made of the number of and the distribution of gall formations with colonies of living insects. The number and distribution of gall formations and the roots of a typical plant for each treatment are shown in figure 2. As may be observed no galls were found near the point of injection. The results indicate that the insecticide, at the 200-pound rate, was effective for a distance of 7 to 10 inches from the point of injection.

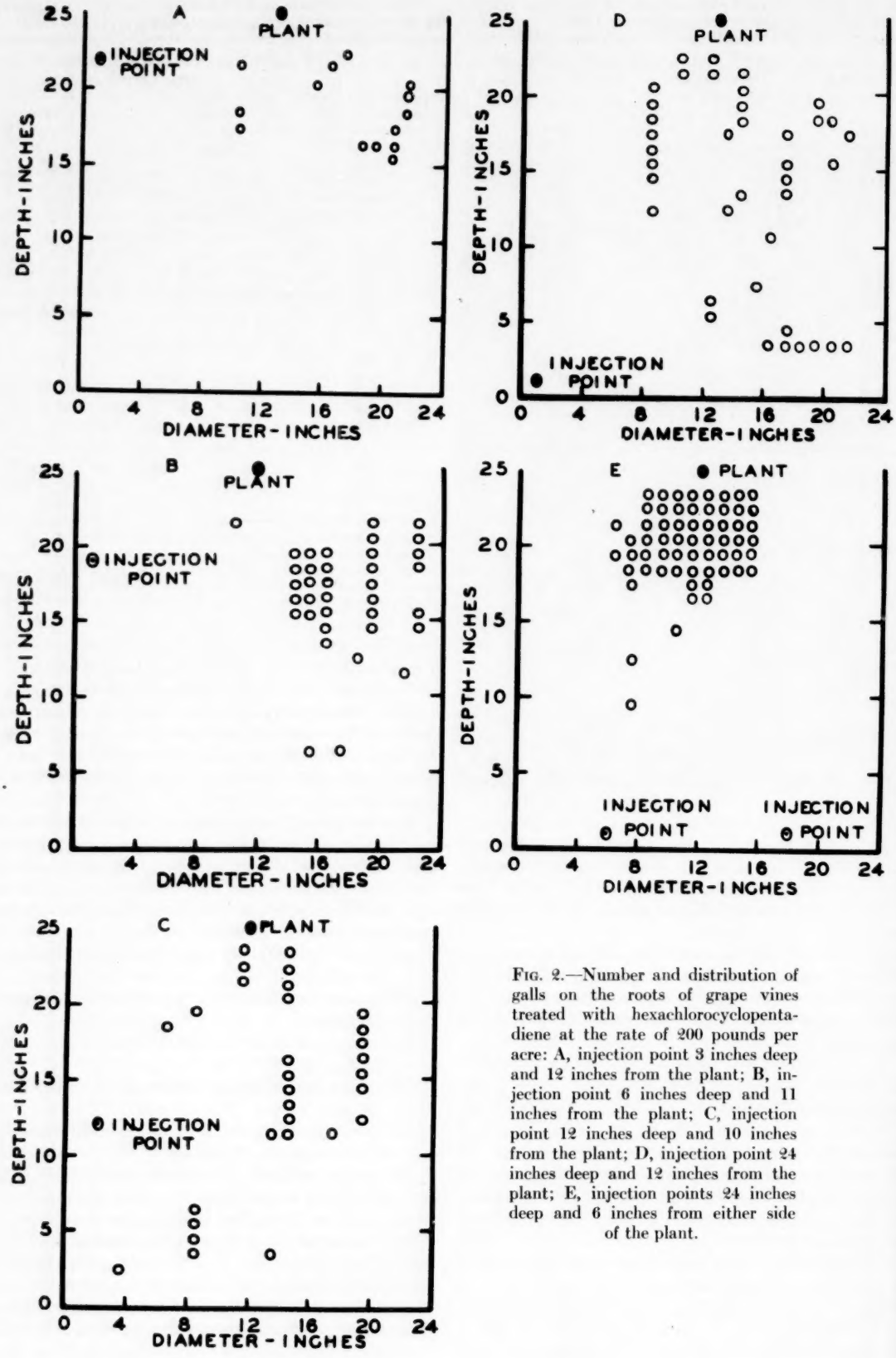

FIG. 2.—Number and distribution of galls on the roots of grape vines treated with hexachlorocyclopentadiene at the rate of 200 pounds per acre: A, injection point 3 inches deep and 12 inches from the plant; B, injection point 6 inches deep and 11 inches from the plant; C, injection point 12 inches deep and 10 inches from the plant; D, injection point 24 inches deep and 12 inches from the plant; E, injection points 24 inches deep and 6 inches from either side of the plant.

The results shown in the previous experiments were further investigated in the fall of 1957. The vines were planted at 12-inch intervals in a single row. The fumigant was injected into the soil on a 12-×12-inch grid and at a depth of 3 and 6 inches. The treatments were replicated two times and each replicate consisted of eight plants. A list of the treatments and a summary of the results are shown in table 2. The results indicate that the material at the 100-, 150-/and 200-pound rates greatly reduced the number of galls on the roots. At the 50-pound rate the material was relatively ineffective.

TESTS IN 1958.—In some of the tests the insecticide was injected into the soil and in others an emulsifiable concentrate was mixed with water and applied to the surface. The plants were seedling grape vines, 2 years old. They were planted at 12-inch intervals in a single row. Each treatment was replicated four times and a replicate consisted of eight plants. A list of the treatments and a summary of the results are given in table 2. The results indicate that surface applications of the material were quite effective against the insect. The material, injected into the soil at a depth of 6 inches, did not give a high degree of control. It should be mentioned that the root system of the grape vines was quite shallow and the insect population was near the surface. This factor may have had some influence on the results of the experiments.

A Comparison of Certain Artificial Diets for Laboratory Cultures of the Pink Bollworm[1]

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ABSTRACT

Artificial diets having purified casein, raw cottonseed meal and a special processed cottonseed meal as their basic ingredients were tested as rearing media for the pink bollworm, *Pectinophora gossypiella* (Saund.). Larvae were reared in individual glass vials under nonaseptic conditions. Three antimicrobial agents, butyl paraben, methyl paraben and sorbic acid were incorporated into the diets as a preventive measure against contamination by microorganisms.

Average duration of the larval stage was 25.6 days on the casein diet, 14.0 days on the processed cottonseed meal and 12.9 days on the raw cottonseed meal. Average pupal weights for both sexes were 13.7 mg., 19.8 mg. and 21.3 mg., respectively, for insects reared on the casein, raw cottonseed meal and processed cottonseed meal diets. The diet-reared pupae were not as large as field-collected ones. Oviposition records indicated that females reared from the raw cottonseed meal diet produced the most and females from the casein diet the least number of eggs.

There was little difference among the diets with regard to the percentage of larvae developing to the adult stage. Best results were obtained with the casein diet as approximately 60% of the larvae placed on the diet achieved maturity.

The pink bollworm *Pectinophora gossypiella* (Saund.) has been successfully reared in the laboratory on several artificial diets. Research pertaining to development of certain diets and to the nutrition of the insect have been reported by Beckman *et al.* (1953), Vanderzant (1957, 1958), Vanderzant & Reiser (1956a, 1956b) and Vanderzant *et al.* (1956, 1957). Although there are several synthetic diets available for laboratory cultures of the pink bollworm, none have been evaluated for relative usefulness in general laboratory rearing.

COMPOSITION OF TEST DIETS.—The casein diet developed by Vanderzant & Reiser (1956) and the cottonseed meal diet developed by Clark (1958) were selected for comparison. A comparison of the ingredients required for each diet is made in table 1.

Two types of cottonseed meal were used in these studies. The type termed in this report as raw cottonseed meal was made from cottonseeds which had the seed coats removed and the meats crushed. None of the oil was removed from this meal. The other type, termed processed cottonseed meal, was similar to the raw cottonseed meal referred to above except that the cottonseed oil and gossypol had been removed by a special solvent process developed by the Cottonseed Products Laboratory of the Texas Engineering Experiment Station. The oil content of the processed meal was below the minimum required for normal development of the insect; thus, 1.5

Table 1.—A comparison of ingredients needed to mix 100 grams each of the casein and cottonseed meal diets.

CASEIN		COTTONSEED MEAL[a]	
Ingredients	Grams	Ingredients	Grams
Casein, vitamin free	5.00	Cottonseed meal	16.25
L-cystine	0.10	Sucrose	2.00
Glycine	0.15	Brewer's yeast	1.50
Sucrose, C.P.	8.00	Alphacel	1.50
Corn oil (Mazola)	1.00	Albumin	1.00
Cholesterol, U.S.P.	0.30	Agar	1.50
Salt mixture, Wesson's	0.70	Wheat germ oil	0.50
Choline chloride	0.10	Cholesterol	0.15
Alphacel	4.00	Water	75.00 ml.
Agar	3.00	Antimicrobial mixture[c]	10.00 ml.
Sodium alginate	0.50		
Vitamin mixture[b]	1.00 ml.		
Water to 100 grams			
Antimicrobial mixture[c]	10.00 ml.		

[a] 16.25 grams of cottonseed meal was used for the raw or the processed cottonseed meal diets. The type of meal was varied according to the diet desired.

[b] Contains 1.0 mg. niacinamide, 1.0 mg. calcium panthothenate, 0.25 ml. thiamine hydrochloride, 0.50 mg. riboflavin, 0.25 mg. pyrixdoxine hydrochloride, 0.25 mg. folic acid, 0.02 mg. biotin, 0.5002 mg. vitamin B_{12} and 20.0 mg. inositol.

[c] Each 10 ml. contained 0.2 grams each of butyl paraben, methyl paraben and sorbic acid dissolved in 95% ethyl alcohol.

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grams of corn oil was added to each 100 grams of this diet in order to satisfy the dietary fat requirements of the larvae.

The use of the antimicrobial agents, butyl paraben, methyl paraben and sorbic acid, in the concentrations reported in table 1 made it possible to rear the pink bollworm by use of nonaseptic techniques. These are the agents that were used by Clark (1958) in his earlier work with the cottonseed meal diet. This procedure greatly facilitated the rearing process by preventing contamination and by reducing labor requirements.

PREPARATION OF DIETS.—Each of the diets was prepared in a similar manner. The dry ingredients were weighed separately on a torsion balance. The agar was dissolved in cold water and heated to the boiling point. The remainder of the dry ingredients were then dumped in and allowed to boil for 2 to 3 minutes. This mixture was then poured into a Waring Blendor. The antimicrobial mixture was pipetted into the blender. The vitamin mixture was added to the casein diet in a similar manner. The hot diets were allowed to blend until they appeared to be thoroughly mixed. The diets were then poured from the blender jar into plastic "squeeze" bottles of the type commonly used for dispensing kitchen condiments. The spout of the bottle was inserted into previously sterilized 2-dram shell vials and the diet dispensed into them. Each vial was filled to about one-third of its volume. During this procedure, the diets had to be handled rapidly as they solidify quickly on cooling. As soon as the hot diets were added to the vials, they were loosely wrapped in heavy paper and allowed to cool until all free moisture on sides of the vials had evaporated. This usually required about 12 hours.

REARING PROCEDURE.—Pink bollworm eggs were collected daily from cotton leaves maintained in standard oviposition cages. These eggs were washed in distilled water and collected in a small vial on a piece of filter paper. The vial was then plugged with cotton. As the young larvae emerged, they would congregate on the sides of the vial and on the cotton plug. The larvae were then transferred by means of a soft camel's-hair brush to vials containing the diets. During the transfer, the brush was moistened frequently in an antiseptic solution containing 3000 p.p.m. of "Roccal" (benzalkonium chloride) as a preventive measure against microbial contamination of the diets. One larvae was placed on the side of each vial just above the diet. As soon as a transfer was accomplished the vial was plugged with nonabsorbent cotton and placed in a rack. The larvae were held until they reached the adult stage in an incubator that maintained 85° F. ± 2° and an approximate relative humidity of 90%. Continuous light was maintained throughout the test period.

BIOLOGICAL RECORDS.—Each vial was numbered in order that records could be maintained on individual insects for the duration of the developmental period. The insects were observed daily and the appropriate records taken.

The average duration of the developmental stages of the pink bollworm on the test diets is presented in table 2. Development was the slowest on the casein diet and the most rapid on the raw cottonseed meal. The average duration of the larval stage was 25.6 days on the casein, 14.0 days on the processed cottonseed meal and 12.9 days on the raw cottonseed meal diet. From egg to adult development ranged from 18 to 37 days on the casein diet and from 10 to 20 days on the raw cottonseed meal. Records for square- and boll-fed larvae as reported by Fenton & Owen (1953) indicated that 10.1 days are required for larval development on squares and 16.5 days are required on bolls. There was little difference in the time required for the prepupal or pupal periods of the pink bollworms reared on the various diets. The duration of the developmental period appeared to about normal on the cottonseed meal diets and slightly longer than normal on the casein.

Table 2.—Average duration, in days, of the immature stages of the pink bollworm when reared on several artificial diets.

Diet	Number of Insects	Larvae	Larval Range	Prepupal	Pupae
Casein	45	25.6	18–37	2.3	7.6
Raw cottonseed meal	65	12.9	10–20	2.3	7.9
Proc. cottonseed meal	59	14.0	12–23	2.6	8.4

Pupal weights are presented in table 3. The male pupae weighed less than the females. The largest diet-reared pupae were collected from the processed cottonseed meal and the smallest from the casein diet. None of the pupal weight averages for diet-reared insects was as great as those for pupae collected from cotton bolls in the field.

Oviposition records were taken from individual female moths reared from the diets and from adults which were reared from larvae collected from field-grown cotton bolls. Single pairs of moths were confined without food or water in small glass-enclosed cages containing one cotton terminal each. The terminals were maintained in small bottles of water. Terminals were replaced every 3 days. When the terminals were removed from the cages, they were carefully examined under a binocular microscope for pink bollworm eggs and number of eggs per female recorded. Results of these tests are presented in table 4.

Females reared on the raw cottonseed meal diet averaged the largest number of eggs per individual and females from the casein diet produced the smallest number of eggs. Egg-production by females obtained from the two cottonseed meal diets were comparable with the egg-production of the field-collected insects. Experience has indicated that egg-production per female can be increased by furnishing the ovipositing moths with a sugar-water solution.

YIELD.—The efficiency of the diets for rearing the pink

Table 3.—Pupal weights of pink bollworms reared on several diets.

Diet	Weights in Milligrams		
	Male	Female	Average
Casein	12.8	14.5	13.7
Raw cottonseed meal	18.6	21.0	19.8
Proc. cottonseed meal	18.7	23.9	21.3
Field[a]	—	—	23.9

[a] Pupae collected from cotton bolls produced in the field.

Table 4.—Oviposition records of female pink bollworms reared on several diets.

DIET	NUMBER OF FEMALES	EGG PER FEMALE	RANGE
Casein	40	10.6	1–40
Raw cottonseed meal	46	45.4	1–88
Processed cottonseed meal	46	26.7	1–73
Field[a]	19	32.2	2–91

[a] Female moths produced by larvae collected from cotton bolls produced in the field.

bollworm was rated according the number of adults recovered when compared with the number of larvae placed on the diets. As indicated in table 5, there was little difference among the diets in this respect. Neither of the diets produced a very good yield of adults. Almost 60% of the larvae placed on the casein and raw cottonseed meal diets completed development while only about 50% reached the adult stage on the processed cottonseed meal.

DISCUSSION.—None of the diets proved very satisfactory for rearing large numbers of pink bollworms for routine laboratory experiments. The unsatisfactory yield of adults on the diets is a prime consideration against their use, except for special purposes. These diets have proved to be very useful for studies on the nutrition of the insect and with respect to factors inducing diapause. However, these media must be held under carefully controlled conditions in order to prevent microbial contamination or drying of the diet, both of which can render them unfit for rearing purposes.

Table 5.—Comparative yields of the diets as indicated by the percentage of pink bollworms to develop to the adult stage on each diet.

DIET	NO. OF BATCHES TESTED	ORIGINAL NO. LARVAE	ADULTS RECOVERED	PER CENT SURVIVAL (YIELD)
Casein	11	1,151	685	59.5
Raw cottonseed meal	37	1,934	1,138	58.8
Processed cottonseed meal	16	1,152	551	47.8

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Certain Factors Influencing Diapause in the Pink Bollworm, *Pectinophora gossypiella*[1]

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ABSTRACT

Larvae of the pink bollworm (*Pectinophora gossypiella* (Saund.)) reared on certain artificial diets were exposed to various environmental conditions in order to study the influence of these factors on the incidence of diapause. Regardless of the diet, exposure to temperatures of 70° F. or lower was sufficient to cause diapause in a high percentage of the larvae tested. The longer the exposure period the greater the percentage of diapausing larvae. A high incidence of diapause occurred when larvae reared on cottonseed meal diets were exposed to either short or abnormally long days. Diapause did not occur with exposure to a typical long day of 14 hours of light. Under these photoperiodic conditions, a greater percentage of diapausing larvae was observed among individuals reared on a 5% oil diet than on one containing 1% oil. No response to photoperiod was obtained in larvae reared on a wheat germ medium. These results indicate that a probable relationship exists between photoperiodism and diet as factors inducing diapause in the pink bollworm. Relative humidity was also found to influence the incidence of diapause in larvae produced on the cottonseed meal diets.

The pink bollworm (*Pectinophora gossypiella* (Saund.)) overwinters in the last larval instar in a state of diapause. A study of the factors influencing the onset of diapause in this insect is important not only from the viewpoint of scientific interest, but it also has practical application. The pink bollworm is an important pest of cotton, capable of causing tremendous financial losses in cotton-growing areas the world over. The principal means for controlling the pink bollworm involves the use of cultural methods. These methods include the complete destruction of cotton stalks which kills the diapausing larvae and also eliminates possible sources of food. Since the control of this insect is based on the elimination of

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large populations of overwintering larvae, any knowledge gained concerning factors which cause the insect to enter diapause could be used in furthering the development of control measures to eliminate the pink bollworm as an economically important pest.

Several authors have advanced theories to explain diapause in the pink bollworm, but in all cases the observations were based on field experiments in which several variables could not be controlled.

Early publications by Willcocks (1916) and Gough (1916) reported the occurrence of diapause in pink bollworm larvae in Egypt. These authors believed diapause to be an aestivation period induced by temperatures encountered by the larvae during the last instar. Larval diet and photoperiod also have been suggested as possible factors which influence the induction of diapause in the pink bollworm. Squire (1937) and Fife (1949) both emphasized the importance of the larval diet in relation to the incidence of diapause. Lukefahr (1959) is currently conducting experiments which indicate that photoperiod has an influence on the incidence of diapause in pink bollworms reared on cotton plants growing under controlled conditions.

The objective of the research discussed in this paper was to isolate certain environmental factors under controlled laboratory conditions and determine their relative importance in the induction of diapause. The pink bollworms were reared on artificial diets.

REARING METHODS.—Moths that were reared in the laboratory on artificial diets were the principal source of eggs used in the experiments. Eggs were collected from standard oviposition cages and passed through a washing tube containing distilled water. The eggs then were collected on a piece of filter paper inside of a small glass vial which was plugged with nonabsorbent cotton and held at 85° F. until the eggs hatched.

Artificial diets, including the wheat-germ diet developed by Vanderzant (1959) and a modification of the cottonseed meal diet developed by Clark (1958) were used in all of the experiments. Insects were reared individually in 2-dram, shell glass vials.

A single 1-day-old larva was placed in each vial with a fine-tipped sable brush. The vials were then plugged with nonabsorbent cotton and placed in the appropriate testing location. Each test was replicated a minimum of four times.

Tests were conducted in rooms or cabinets in which temperature could be regulated to within ±2° F. Relative humidity was controlled to within ±5% and the photoperiod was regulated as desired.

An aseptic rearing technique was not used as antimicrobial agents were added to each diet to inhibit growth of molds, yeasts and bacteria. The chemicals used were sorbic acid, methyl para-hydroxybenzoate and butyl para-hydroxybenzoate. Sufficient amounts were added to attain a concentration of 0.2% by weight of each chemical in the diet. Vials that became contaminated were discarded and not considered in the final observations.

DETERMINATION OF DIAPAUSE.—For the purpose of these experiments, diapause was considered as the resting stage entered into by the larvae which, once initiated, persisted even under conditions favorable for pupation. Certain criteria were established to determine if an individual larva was in a state of diapause. Extensive rearings established the optimum conditions for normal development of the pink bollworm. Diapause rarely occurred with constant temperatures of 80° F. or 85° F., high relative humidity (80% to 95%) and continuous light. In experiments involving the use of temperatures lower than 80° F., suspected long cycle larvae were held in the testing location 2 or more weeks beyond the time when pupation was last observed among the individuals of a particular experimental group. Larvae remaining after this period were then placed in an 85° F. environment and held an additional 2 weeks. Larvae which had not pupated by the end of this period were classified as diapausing. When possible, the remaining larvae were analyzed for fat content and the results compared with the percentage of fat content previously determined for diapausing and nondiapausing larvae collected in the field. Fat analyses were conducted with a micro-soxhlet extraction apparatus and petroleum ether solvent.

In photoperiod and relative humidity experiments conducted at optimum rearing temperatures, remaining larvae were held in the testing location 2 or more weeks beyond the date when pupation was last observed among individuals tested. Remaining larvae at this examination were considered to be in diapause.

In all cases, tests were not terminated until all of the larvae concerned had attained the final instar and had remained in this stage well beyond the time normally required for pupation. In the temperature experiments, all of the diapausing larvae remained for at least 1 month or more in the testing location after attaining the final larval instar.

TEMPERATURE EXPERIMENTS.—Several experiments were conducted to determine the influence of temperature variations on the initiation of diapause in the pink bollworm. Wheat germ was used for rearing all insects in the tests herein reported. Each test was conducted under conditions of continuous light and high (90% to 95%) relative humidity.

When pink bollworm larvae were exposed each day of larval life to various periods of both cool and warm temperatures, the results indicated a definite influence of temperature on the induction of diapause (table 1). Larvae maintained continuously during the entire developmental period at 90° F., 85° F., and 80° F. temperatures did not enter diapause. Temperatures lower than 80° F. were sufficient stimuli to induce diapause in the larvae.

Results also indicate that a variation of 10° F. within the range of temperatures optimum for growth and development (80° F. to 90° F.) does not induce diapause. Daily variation of 10° F. can induce diapause in the pink bollworm provided the fluctuation is on the minus side of 80° F. An even greater response to temperature was observed among individuals exposed to a 20° F. decrease from the optimum rearing temperature of 85° F. Results further indicated that the longer the larvae are held at the lower temperatures each day the more likely they are to diapause.

Table 2 presents the results of an experiment designed to relate the incidence of diapause to the duration of the larval developmental period spent in a cool environment. A large number of vials of the wheat-germ diet contain-

Table 1.—Effect of varying temperature exposures on the incidence of diapause in pink bollworms reared on wheat-germ diets.

DAILY TEMPERATURE TREATMENT (° F.)	NO. OF INSECTS	DIAPAUSING LARVAE (%)
Continuous 90	120	0.0
Continuous 85	286	0.0
Continuous 80	114	0.0
Continuous 75	130	42.3
Continuous 65	88	100.0
16 hrs. 80— 8 hrs. 90	333	0.0
16 hrs. 85— 8 hrs. 75	402	6.1
8 hrs. 85—16 hrs. 75	355	17.4
16 hrs. 85— 8 hrs. 65	462	18.0
8 hrs. 85—16 hrs. 65	198	76.9

ing one 1-day old larva each were prepared and placed in a cabinet maintaining a constant temperature of 85° F. Each day thereafter, 30 larvae were removed and reared for the remainder of the testing period at 70° F. This daily transferal was continued until the individuals remaining in the initial group at 85° F. began to pupate. Results indicate the percentage of diapausing individuals was influenced by the age of the insects on transferal to the cooler environment. All the larvae that were held for only 1 or 2 days in the warm environment prior to transfer to the lower temperature entered diapause. Insects that spent their entire developmental period at 85° F. did not enter diapause. Incidence of diapause evidently was influenced by the length of the larval period that was spent in the cool environment. The lon er a group of larvae was allowed to remain in the warm environment before being transferred to the cooler environment, the smaller the percentage of individuals that entered diapause. As the data indicate, the longer a larva was exposed to cool temperature during the first 18 days of larval development, the more likely it was to enter a state of diapause. The reaction of the pink bollworm larvae to alternating periods of cool and warm temperatures is similar to that obtained by Masaki (1957) in work with *Barathra brassicae* (L).

Included in table 2 are the results of fat analyses based on dry weight of the diapause larvae occurring in each age group. The results are consistent with analyses conducted on diapausing larvae collected in the field. In all of the diapausing larvae, approximately 40% of the dry body weight consisted of fat.

In the same experiment, records of the duration of the immature stages of the pink bollworm were obtained and are given in table 2. The number of days required to complete larval development was related to the time that was spent at 70° F. Insects that spent all but the first 2 days of larval life at 70° F. required an average of 58 days to attain the pupal stage. On the other hand, insects that spent the entire developmental period in an 85° F. environment required only 21 days to reach the pupal stage. The longer an insect was allowed to develop at 85° F. prior to transferal to 70° F., the shorter was the larval developmental period.

The influence of continuous exposure to low temperature (65° F.) was studied. Beginning with the first day of larval life, different groups of insects were exposed for periods of 3, 5, 7, 9, 11, 15, 20, and 25 days, respectively. Following the exposure period, each group was allowed to complete development at 85° F., 95% relative humidity and continuous light. As the number of days of exposure to 65° F. increased, the percentage of diapausing insects also increased (table 3).

Similarly, as the number of days exposure to 65° F. increased, the length of the developmental period increased. Larvae exposed to the cool temperature for only 3 days and then allowed to complete development at 85° F. required 28 days to reach the adult stage. Insects that remained at 65° F. for 25 days prior to transferal required 45 days to reach the adult stage, an increase of 17 days.

PHOTOPERIOD EXPERIMENTS.—Groups of pink bollworm larvae reared on wheat-germ diets were exposed each day to the following conditions: (1) continuous light, (2) 8 hours of light and 16 hours of darkness, (3)

Table 2.—Percentage of diapausing larvae, duration of developmental periods, and fat contents of pink bollworms removed from an 85° F. environment at different periods during larval development and reared for the remainder of the larval developmental period in a 70° F. environment.[a]

AGE IN DAYS WHEN TRANSFERRED	NO. OF INSECTS	DIAPAUSE (%)	AVERAGE NO. OF DAYS FOR LARVAL DEVELOPMENT[b]	FAT CONTENT (%)
1	39	100.0	All diapause	46.2
2	49	100.0	All diapause	40.1
3	66	90.9	58	40.7
4	72	91.7	56	40.2
5	92	92.4	60	39.6
6	104	85.6	51	40.4
7	96	86.5	45	40.0
8	107	71.0	36	40.6
9	111	72.1	35	39.5
10	116	59.5	31	42.2
11	115	33.0	31	41.2
12	118	28.0	27	39.3
13	120	22.5	23	40.0
14	118	13.0	23	—
15	119	11.8	23	—
16	89	9.0	22	—
17	60	9.0	22	—
Control (85° F.)	228	0.0	21	—

[a] All of the insects were held in the 70° F. environment for at least 2½ months after transferal.

[b] This average represents only the larvae that pupated during the experimental period.

Table 3.—Percentage of diapausing larvae and length of developmental period of pink bollworms exposed to 65° F. for varying period when reared on wheat-germ diet.

NO. OF DAYS EXPOSED TO 65° F.[a]	NO. OF INSECTS	NO. OF DAYS TO 1ST ADULT	PER CENT DIAPAUSE
3	184	28	2.1
5	192	28	0.0
7	188	28	4.3
9	136	30	5.9
11	194	35	6.7
15	158	39	8.9
20	152	39	13.2
25	124	45	11.3

[a] Insects were placed in the 65° F. room on the first day of larval life. After exposure to 65° F., the insects were held at 85° F. until development was completed.

Table 4.—Effect of certain photoperiods on percentage diapause in pink bollworms reared on wheat-germ diets.

Hours		No. of Insects	Percentage Diapause
Light	Dark		
24	0	429	0.0
8	16	442	0.5
12	12	425	1.1
16	8	429	0.0
0	24	472	0.6

12 hours of light and 12 hours of darkness, (4) 16 hours of light and 8 hours of darkness, and (5) continuous darkness. The experiments were conducted at 80° F. and light was provided by "daylight" neon tubes. None of these conditions had an influence on the incidence of diapause (table 4). Very few larvae diapaused at any photoperiodic treatment.

In another test, two groups of pink bollworms reared on wheat-germ diet were exposed respectively to increases and decreases in photoperiod of 15 minutes per day during larval development. The experiments were initiated with light-dark conditions of 12 hours of light and 12 hours of darkness. The results of this experiment indicated that neither treatment had an important influence on the incidence of diapause. When the photoperiod was increased, only 1.5% of the larvae entered diapause. When the photoperiod was decreased, only 1.0% of the larvae entered diapause.

Further investigations of the influence of photoperiod on the induction of diapause were conducted with pink bollworms reared on a cottonseed meal diet. The oil was extracted from the cottonseed meal used to prepare this diet. This procedure allowed the addition of known amounts of oil to the diet during preparation. Dietary oil concentrations of 1% and 5% were used to provide diets containing relatively low and high oil content. Groups of insects were exposed to alternating photoperiods per day as follows: (1) 9 hours of light and 15 hours of darkness, (2) 10 hours of light and 14 hours of darkness, (3) 14 hours of light and 10 hours of darkness and (4) 19 hours of light and 5 hours of darkness. Results of this experiment showed a definite indication that photoperiod influences the induction of diapause (table 5). The data indicated that diapause in pink bollworm larvae reared on both the high- and low-oil diets reached a maximum at the shorter light periods. Under conditions of 9 hours of light and 15 hours of darkness, 94.2% of the individuals reared on the 5% oil diet entered diapause and 68.3% of those on the 1% oil diet diapaused. With light-dark periods of 10 hours of light and 14 hours of darkness, 91.5% of the individuals on the 5% oil diet entered diapause and 48.6% of those on the 1% oil diet were in diapause. At light-dark periods of 14 hours of light and 10 hours of darkness, no diapausing larvae occurred at either dietary oil level. At a long light period of 19 hours of light with 5 hours of darkness, diapause percentage had increased to 64.0% in larvae reared on the high-oil diet and 41.5% in larvae on the low-oil diet.

Table 5.—Effect of different photoperiods on diapause in pink bollworms reared on processed cottonseed meal diets containing high (5%) and low (1%) oil contents.

Hours		No. of Insects		Percentage Diapause	
Light	Dark	High-Oil	Low-Oil	High-Oil	Low-Oil
9	15	154	145	94.2	68.3
10	14	153	138	91.5	48.6
14	10	147	121	0.6	0.0
19	5	147	147	64.0	41.5

Results obtained in the photoperiod experiments clearly indicate that larval diet is a critical factor in the response of the pink bollworm to photoperiodic conditions that influence diapause. Pink bollworms reared on the wheat-germ diet did not respond to photoperiod, while those insects reared on cottonseed meal showed a striking response to photoperiodic variations.

Dryness of Larval Food.—Pink bollworms were reared in vials at varying relative humidity levels to determine any influence on diapause. Tests were conducted in battery jars where the relative humidity could be controlled by different concentrations of sulfuric acid. The wheat-germ and raw cotton-seed meal diets were used for these experiments.

Results indicated that the influence of relative humidity on the incidence of diapause depended on the type of diet on which the insects were feeding (table 6). On the wheat-germ diet, there were only slight differences in the percentages of diapausing larvae at the different relative humidity levels. At each relative humidity level only 5% or less of the larvae entered diapause. A definite response was noted at each relative humidity level among larvae feeding on the cottonseed meal diet. Almost 60% of the insects reared at the 0% relative humidity level entered a state of diapause, while only 30% of the larvae entered diapause at the 100% relative humidity level.

Seasonal Influence.—Tests were conducted from late April to December, 1959 to determine the influence of seasonal environmental changes on the incidence of diapause in the pink bollworm.

Each week, 288 vials of wheat-germ diet containing one 1-day-old larvae each were divided among four racks each with 72 vial capacity. Two racks were each carefully wrapped with dark cloth to exclude all light; the other two racks were left exposed. One set of the wrapped and one set of the exposed vials were placed in an open insectary

Table 6.—Effect of different relative humidity levels on diapause in pink bollworms reared on wheat-germ and raw cottonseed meal diets.[a]

Type of Diet	Relative Humidity (%)	No. of Insects	Diapause (%)
Wheat-germ	0	118	5.0
	60	125	3.2
	80	113	3.5
	100	103	4.9
Cottonseed	0	149	59.8
	60	149	48.7
	100	154	29.9

[a] All tests were conducted in a room under standard conditions of 80° F. and continuous light.

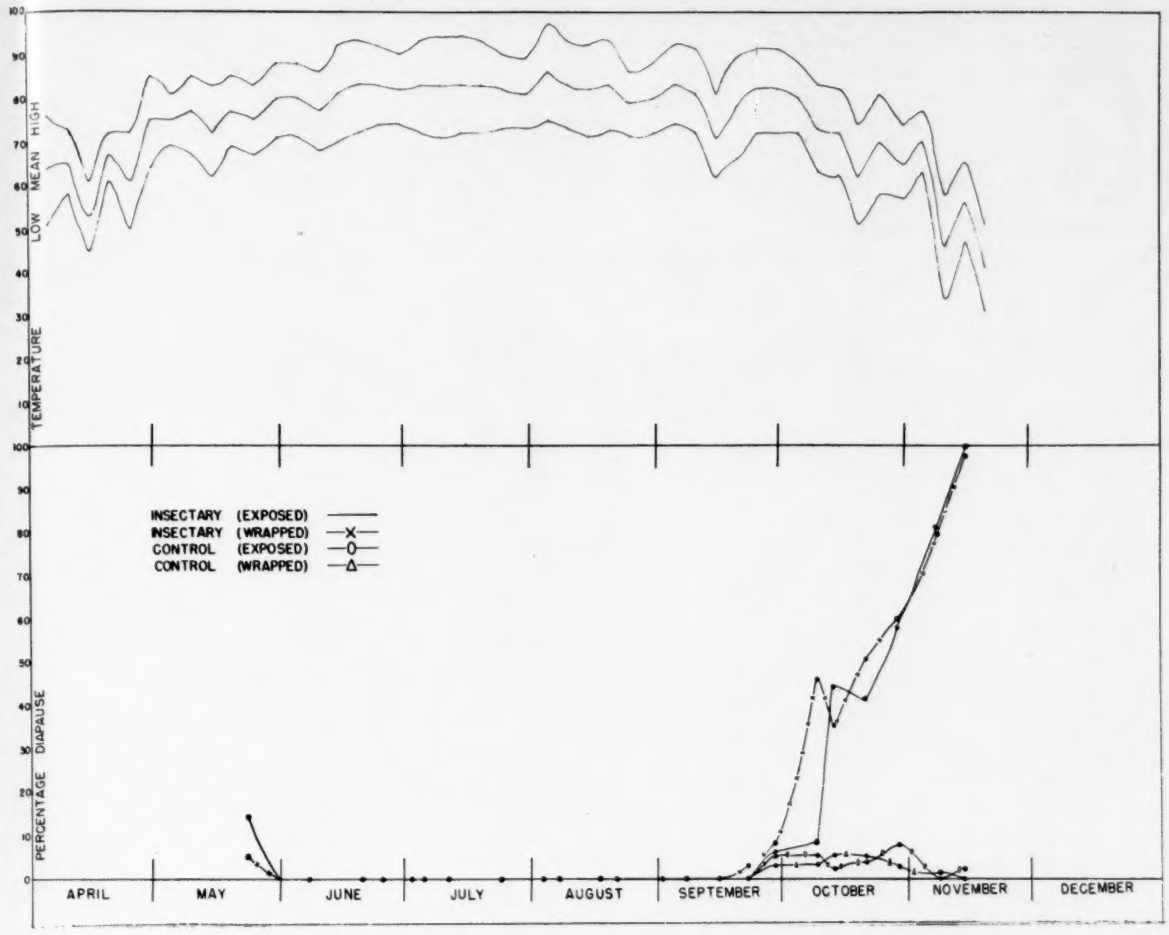

FIG. 1.—The seasonal occurrence of diapause in pink bollworm larvae reared on the wheat germ diet when held in the laboratory and in an open insectary.

where they were exposed to all effective environmental changes except rainfall and direct sunlight. The remaining two sets of vials were placed in a rearing room where optimum conditions for development were maintained. The control insects were exposed to natural light variations. Results of this experiment are presented in figure 1. A small number of diapausing individuals appeared in the first group of pink bollworms that were placed in the insectary in late April. This occurrence of diapause may be attributed to the cool temperatures persisting at that time. Almost 4 months passed before diapausing larvae appeared in either the insectary or control group. With the cooling temperature of early fall, diapause increased in the insectary tests at a rapid rate. In a 6-week period, the percentage of diapausing larvae increased from 0% to 100% and remained at that level until the tests were terminated in December. At the same time, a small percentage of diapausing larvae was appearing in the control group. This occurrence of diapause in the control group may have been owing to some influence by photoperiod or unknown stimulus. Temperature was apparently the dominant factor influencing the incidence of diapause in the insectary-reared insects. Percentage diapause increased at a comparable rate among larvae exposed to light. Insects exposed to light reacted to the temperature stimulus about a week earlier than those protected from light.

DISCUSSION AND CONCLUSIONS.—In these experiments, temperature proved to be the one factor which could be adjusted to cause an appreciable number of pink bollworm larvae to enter diapause regardless of the type of diet that was used. Photoperiod and relative humidity variation induced diapause in a large percentage of the larvae reared on cottonseed meal diets, but proved less effective in those reared on wheat-germ diets. Only low temperatures (65° F. to 75° F.) produced diapausing insects on the wheat-germ diet, thus indicating that under laboratory conditions temperature may be the dominant factor inducing diapause.

A short day length induced diapause if the pink bollworm larvae were reared on a diet generally comparable to the natural food material. The results obtained with pink bollworms agree very well with observations of the photoperiodic influence of diapause in a number of other species of arthropods. For example, the general pattern of photoperiodic response was similar to the oriental fruit moth, *Grapholitha molesta* (Busck) (Dickson 1949) and the European red mite, *Panonychus ulmi* (Koch) (Lees

1953), in that short or medium light periods induced diapause. In evaluating the action of light as a factor influencing the induction of diapause in the pink bollworm, the larval diet apparently must be considered as a contributory factor in photoperiodic responses. Very few diapausing individuals appeared among larvae reared on the wheat-germ diet regardless of the photoperiodic treatment. The appropriate light-dark combinations could either induce or prevent the occurrence of diapausing individuals among larvae reared on either the high- or low-oil cottonseed diets. It was significant, however, that the higher percentage of diapausing individuals was always found on the high-oil diet regardless of the light-dark combination used. Results of seasonal tests with insectary-reared pink bollworms closely approximated the results recorded for pink bollworms grown in the field. Very few diapausing larvae appeared in the tests until September, when cooler temperatures began to occur. Percentage diapause increased rapidly through October and reached a maximum of 100% in November. In Central Texas, Fife *et al.* (1957) found that only 11% of the larvae feeding on green bolls in July and August entered diapause whereas 93% entered diapause in the mature bolls in October and November.

The results obtained in this study indicate that diapause in pink bollworms in the field is probably not caused by any single factor but more likely is caused by a complex interaction of several factors. The maximum percentage of diapausing larvae occurs locally at a time of the year when all of the factors that were investigated in the laboratory could be exerting a combined influence. The maximum numbers of diapausing larvae are found in Central Texas during the months of October and November. At this time of the year, the cottonseeds in which the pink bollworm larvae are feeding have reached the maximum percentage of oil content and minimum amount of moisture (Caskey & Gallup 1931). Also, at this time the hours of daylight have shortened considerably and temperatures might not be cool enough to exert a maximum influence on diapause, but in conjunction with the short day lengths and the high oil content of the larval diet, a high incidence of diapause might result from the combined influences. In areas where temperatures are moderate to high the year around, diapause possibly could be induced by the interaction of photoperiod and a rich, dry larval diet.

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Quantitative Measurements of Damage by the Greenbug, *Toxoptera graminum*, to Four Wheat Varieties[1]

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Abstract

It was found that a high infestation of greenbug, *Toxoptera graminum* (Rond.), caused a maximum 55% reduction of wheat plant root systems. Measurements made, to determine at what level of infestation this damage became detectable, were: leaf length gain during infestation, dry leaf weight, dry root weight, and final greenbug count. A varied initial infestation of parental greenbugs plus progeny were maintained on individual plants of Pawnee, Ponca, Bison, and Dickinson wheat. For each criterion measured, the first significant difference between check and infested plants on the several varieties generally occurred at different infestation levels.

Dry root weight showed a maximum loss of from 32% to 55% compared with the control and was the only measurement in which a susceptible winter wheat, Bison, showed less damage than the resistant spring selection, Dickinson. The results indicate that the root systems and above-ground plant parts were approximately equally damaged.

Previous studies concerned with the effects of the green bug, *Toxoptera graminum* (Rond.), have dealt primarily with the visible chlorotic manifestations. Fenton & Whitehead (1944) noted degrees of greenbug

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damage and observed that these could be used to classify potentially resistant plants. Dahms *et al.* (1955) reported differences in varietal reaction to greenbug infestation. Chatters & Schlehuber (1951) studied the feeding site and stylet formation. Painter (1956) summarized the studies of aphid resistance in crop plants. Matthew (1953) studied the relationship between classes of fall greenbug injury to wheat plants in the field in the absence of a spring infestation and the resulting ability of the plants to tiller and produce grain at harvest. Plants sprayed with an aphicide in the fall consistently produced more grams of wheat per surviving plant than the untreated control plants of the same class. The average increase in yield of treated over untreated control for each class was: Class 0 (no injury), 62%; Class 1, 157%; Class 2, 45%; Class 3 (severe injury), 4%. Plants treated in the fall also produced consistently more tillers than plants of the same classification in the untreated control. This study showed that there was a critical class of injury even in the fall beyond which there was little probability of plant recovery. The information reported in the following study partially explains the field results obtained by Matthew.

The initial purpose of this research was to determine if greenbugs feeding on the upper portion of wheat plants might have an adverse effect on the root systems. Preliminary experiments showed that when a plant was exposed to a heavy infestation, there was a marked reduction in root development. A further study was therefore proposed to determine the level of infestation at which damage to the roots or leaves first became measurable in several wheat varieties and to determine if there was a differential varietal reaction based on this and other selected criteria. More detailed records of the experiment reported here are on file at Kansas State University (Ortman 1957).

MATERIALS AND METHODS.—Greenbugs from a culture maintained on Reno barley for several years in the insectary were used. The tests were conducted in a basement insectary with fluorescent lights automatically timed for a 16-hour day. The temperature was maintained at 70° F.

Bison, Pawnee, Ponca, and Dickinson were the wheat varieties used. Dickinson is a greenbug-resistant spring wheat selection; the others are greenbug-susceptible winter wheats.

Individual wheat seeds of approximately the same weight (to the nearest milligram) were planted in 5″ clay pots containing a finely screened soil mixture of 10 parts sand, 4 parts black loam, and 1 part sheep manure. This soil type was used to facilitate root washing at the termination of the test. The plants were infested in the two-leaf stage with 2, 4, 6, 8, and 10 one-day-old alate adults which were permitted to feed and reproduce for 7 days. Each infestation level was replicated four times. A second set of experiments in which the duration of the infestation was varied at five regular intervals from 2 through 10 days with a uniform infestation of 10 aphids was also completed. One-day-old alate adults were obtained by placing nymphs with wing pads in a holding cage and 24 hours later selecting for use those that had fully developed wings. This was done to limit the variability resulting from insect age and also to simulate field conditions since a field infestation usually originates from winged forms.

Four criteria were used to evaluate plant damage: (1) the gain in total leaf length during the period of infestation; (2) final greenbug count which included adults plus progeny; (3) dry leaf weight; and (4) dry root weight. At the termination of the experiment the entire plant was washed, the roots severed from the top at the crown and placed in individual boxes to be dried. The roots and tops were dried in an oven at 72° C. ±2° until they reached constant weight. They were then weighed to the nearest 0.01 milligram using a Roller-Smith precision balance.

RESULTS.—*Dry Root Weight.*—Figure 1 illustrates the differences in dry root weight of the four wheat varieties. The injury was greatest to Ponca and Pawnee and was of approximately the same magnitude in both varieties. There was a marked adverse effect on plants of these two varieties at the lowest infestation level of two parental greenbugs as compared with the uninfested check. A plateau then developed at the successively higher infestation levels. Ponca exhibited another significant root weight reduction at the initial infestation level of eight adults as compared with two adults.

In contrast to the marked loss in root weight by these two varieties there was a nonsignificant increase in the case of Bison and Dickinson at the lowest initial infestation level. Each successively higher infestation level then caused an additional loss in Bison root weights. The highest level of 10 parental greenbugs did not effect a significant reduction in weight as compared with the uninfested check. In Dickinson the first significant reduction was recorded for the initial infestation of four parental greenbugs. Another significant decrease occurred when 10 parental greenbugs were used. On a percentage basis, Bison lost less weight than Dickinson at the comparable infestation level.

The maximum per cent root weight loss compared with the uninfested check was 55% for Pawnee; Ponca, 53%; Bison, 32%; and Dickinson, 44%. Further varietal differences are noted when comparing with the uninfested check plants.

In the varied infestation-period experiments, the overall pattern was similar to that recorded above. The results in that series were more erratic owing to the nature of the experiment which necessitated a series of check plants grown with each infestation period interval. These check plants showed considerable individual variability. In the varied infestation level experiments all levels were compared with the same set of uninfested checks and this minimized the variability of the controls, and made it easier to detect differences.

Final Greenbug Count.—Reproductive potential was influenced by varietal differences (fig. 1). As might be expected the final count increased proportionally with the increased initial infestation but within limits. Each successively higher initial infestation level through six caused a significant progeny increase on Pawnee, and a peak multiplication rate for 7 days occurred when using between six or eight parental greenbugs. The decrease in number of progeny was quite pronounced for 10 parental greenbugs as compared with eight. Again a similar pattern prevailed in the case of Ponca except that a definite peak was recorded for eight parental greenbugs and the reduction for 10 parental was more pronounced.

Lower initial infestation levels produced comparatively fewer progeny on Bison and Dickinson than on Pawnee and Ponca. As in Ponca the peak multiplication rate on

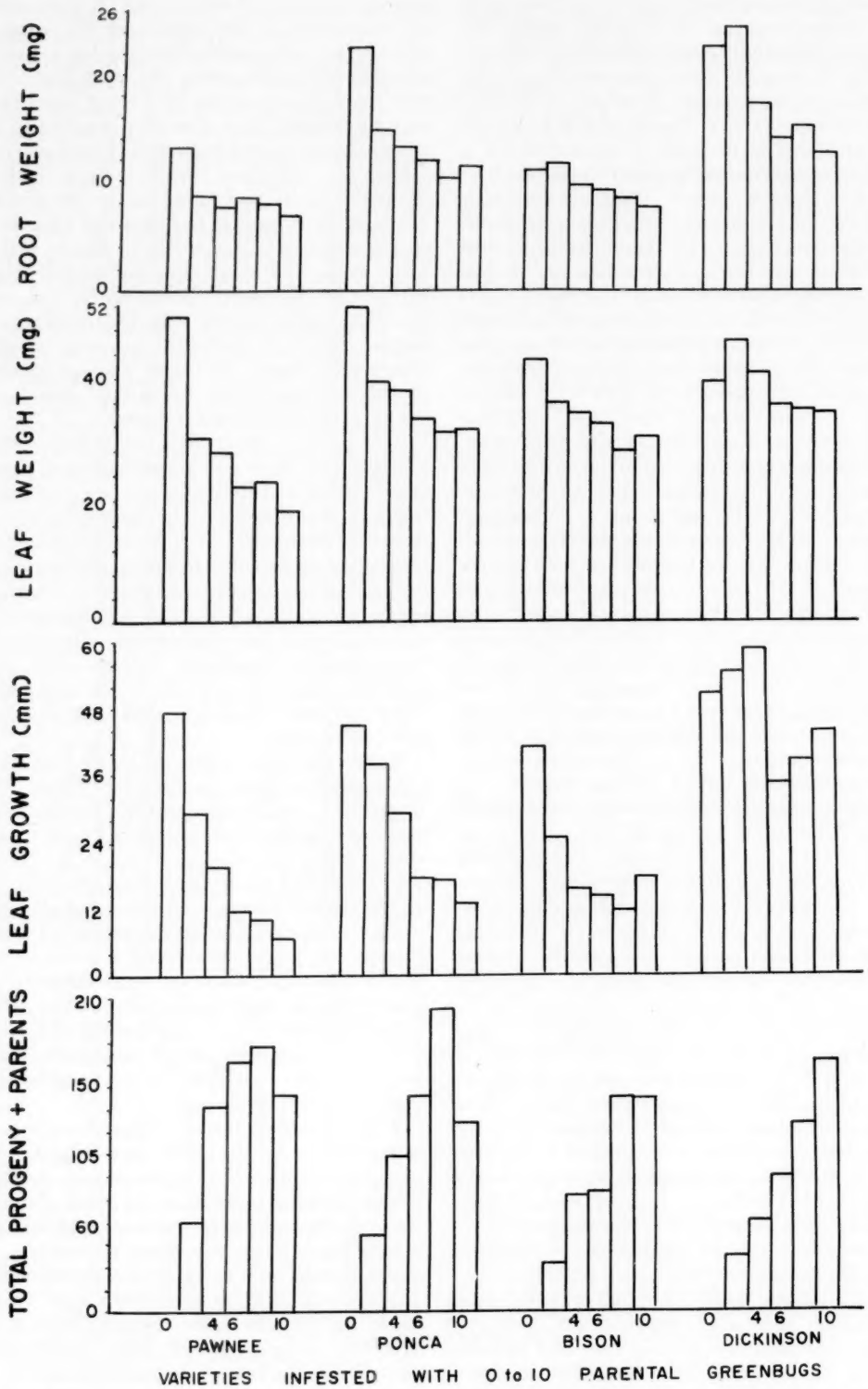

FIG. 1.—Various evaluations of damage to roots and leaves of four wheat varieties after 7 days when initially infested with 0, 2, 4, 6, 8 and 10 adult greenbugs (top three); and the relative parental greenbug fecundity.

Bison appears to occur with eight parental greenbugs, however, the sharp decline with 10 parental greenbugs was not evident in the latter. Greenbugs on Dickinson showed an almost linear response with increased infestation level.

Dry Leaf Weight.—As in dry root weight, leaves of Pawnee and Ponca were most severely damaged and very markedly so at the lowest infestation level (fig. 1). There was a significant weight loss with an initial infestation level of six as compared with two or four but the successively higher infestation levels above six did not produce a significant decrease in Pawnee. On Ponca the reduction in leaf weight was similar for two and four greenbugs, for four and six and for all levels subsequent to six; but six produced a greater loss than two parental, and eight and 10 produced a significantly greater loss than four parental greenbugs.

The lowest infestation produced a significant reduction in Bison dry leaf weight though the percentage loss sustained was not nearly so great as it was with Pawnee and Ponca. Eight parental greenbugs on Bison produced a significantly greater reduction than four.

Contrary to the other varieties, Dickinson exhibited a significant increase in dry leaf weight at the initial infestation level of two parental greenbugs compared with the uninfested check. A significant reduction in leaf weight was recorded for only the highest infestation level. The maximum per cent leaf weight lost was recorded for only the highest infestation level. The maximum per cent leaf weight reduction compared with the uninfested check for Pawnee was 65%; Ponca, 47%; Bison, 33 %; and Dickinson, 14%.

Leaf Length Gain.—Pawnee and Bison were severely stunted by the feeding of two parental greenbugs plus their progeny (fig. 1). The successively higher infestation levels produced evidence of additional stunting.

A significant retardation in growth was first noted on Ponca with four parental greenbugs. Six effected a considerably greater retardation than four but thereafter the decreases were nonsignificant. As in other characteristics measured, there was a nonsignificant increase at the lower infestation levels on Dickinson. The degree of stunting at the three highest infestation levels was similar and was significantly different from the uninfested check at six and eight parental aphids. Compared with the uninfested check the maximum per cent reduction in leaf length gain was: Pawnee, 85; Ponca, 72; Bison, 73; and Dickinson, 33. This measure of injury was probably the most variable one and thus the least reliable.

DISCUSSION.—Pawnee, Ponca, and Bison showed a similar pattern of proportional increase in the rate of aphid reproduction at the lower initial infestation levels and a reduced rate at the high level. This latter effect may have been caused by a depletion of available plant food materials. Restlessness owing to overcrowding may also have been a factor as many greenbugs were observed wandering around the sides of the cages. This decrease in total number of aphids was not evident on Dickinson. There was a nearly linear response in total aphids, which was a measure of reproductive rate, for each increase in initial infestation level. Dickinson was also less chlorotic than any other variety having a comparable number of greenbugs.

The over-all damage pattern as measured by the three criteria was generally similar. However, there were several marked deviations which lead one to suspect that more than a single plant characteristic was responsible for the expression of these damage criteria. Pawnee was significantly damaged, as measured by the three criteria, at the lowest infestation level. Ponca root and leaf weight decreased significantly at the lowest infestation level; however, the leaf stunting was not significant at that level. Bison was stunted and had a reduction in leaf weight at the lowest infestation level; yet, the reduction in root weight was not significant even at the highest infestation level. Dickinson, the resistant spring wheat, showed an increase in all measurements at the lowest infestation in contrast to the reductions in other varieties. This increase was significant only in the case of leaf weight. Significant reduction in root weight was observed first with four parental greenbugs while stunting and reduced leaf weight became significant when six parental greenbugs were used. Since all other varieties used were fall wheats and Dickinson the only spring wheat it might be questioned whether the differences were influenced more by this characteristic or principally by the inherent resistance of Dickinson.

It seems logical that there might be a close relationship between the dry weight of leaves and stunting. However, there were a number of deviations in which at successive levels the value for these criteria was not parallel. This leads to the postulation that the feeding of greenbugs must also in some way alter the metabolism and composition of the host plant.

Greenbug feeding reduced the green plant color. This probable change in plant chlorophyll would impair the efficiency of plant metabolic processes and may explain the apparent discrepancy between stunting and dry leaf weight. For example, the graphs show a considerable difference between stunting and leaf dry weight.

Dry root weight was the only measurement in which a susceptible variety, Bison, was damaged less than the resistant Dickinson. In fact, a significant reduction in root weight in Bison did not occur at any infestation level. There was a nonsignificant increase at the lowest infestation level. This increase in Bison and a similar increase in Dickinson warrant further study. It appears that some type of stimulation may be involved.

Several visual root conditions were observed to contribute to the weight reduction. The roots of infested plants had fewer root hairs and adventitious roots. These roots were also shorter than those of the uninfested check.

Further, it was apparent that not all varieties were equally affected when infested with a comparable number of aphids. It is possible that the varieties showing less injury were more tolerant than others.

Greenbug outbreaks frequently occur in times of limited moisture. The adverse effect on root elongation and total root surface, which was an indirect result of greenbug feeding, must influence the plant's capacity to take up moisture and nutrients. This effect would be compounded in seasons of limited rainfall. Finally, the reduced root system of infested plants would very likely make them more susceptible to winter killing and lower the yield especially during prolonged severe drought periods.

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Tests with Compounds Affecting House Fly Metabolism[1]

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ABSTRACT

Two hundred compounds were tested for ability to sterilize or otherwise interfere with the normal development of house flies (*Musca domestica* L.). Seventy-nine had some deleterious effect when added to the larval medium, but only 10 affected development when combined with the adult food. Amethopterin (Methotrexate®; *N*-[*p* {(2,4-diamino-6-pteridylmethyl]methylamino} benzoyl) glutamic acid), the most active compound, caused sterility in the females but not in the males, in a single feeding when given in the initial meal, and reduced subsequent oviposition when given in a later meal. Oviposition of house flies maintained in a room with a choice of treated and untreated food was only 2% as high as that of flies given only untreated food.

Resistance to chlorinated hydrocarbon insecticides in house flies (*Musca domestica* L.) is now worldwide, and reports of failures to maintain control with organophosphorus compounds are becoming more and more prevalent (Brown 1958). Other groups of compounds, principally the carbamates, have given some promise of restoring the degree of control previously enjoyed, but resistance to these insecticides also may follow prolonged use. Possible methods of control other than those with insecticides acting in the conventional manner should therefore be considered. Among such methods is the use of compounds which have low acute toxicity but which disrupt the normal metabolism and development of the flies.

The startling results obtained in the eradication of the screw-worm (*Callitroga hominivorax* (Cqrl.)) through the release of males sterilized by irradiation (Bushland *et al.* 1951, Baumhover *et al.* 1955, Knipling 1955) have directed attention toward the possibility of controlling other species by this method. Unfortunately, the dense populations of house flies appear to preclude the introduction of greater or equal numbers of sterilized insects, but if a practical chemosterilant could be found and made available to the flies, the insects within the existing wild population would themselves act as agents toward their reduction and eventual elimination. Research in this field, particularly in the retardation of ovarian development, has been conducted by Mitlin *et al.* (1954, 1957), Konecky & Mitlin (1955), Mitlin (1956), Goldsmith *et al.* 1948, 1952, and Goldsmith 1955.

In April 1958 a screening program was initiated at the Orlando, Fla., laboratory in search of compounds acting as sterilants or disrupting normal metabolism and development in other ways. The compounds were introduced into the larval medium and the food of the adult flies. Compounds that were effective in the screening tests were more fully evaluated. This paper describes the screening tests and presents the results with the more promising compounds among the first 200 tested.

METHODS.—Larvae were exposed to two concentrations of the compounds. Fifty grams of dry larval medium was saturated with 100 ml. of water containing 0.1 or 0.5 gram of the chemical in suspension. The treated medium was then placed in a pint jar, 100 eggs were added, and the jar was placed in an emergence cage. Jars containing untreated medium were used as checks. Four days later the medium was examined to determine whether the eggs had hatched and to detect larval abnormalities or mortality. The jars were then left undisturbed until the adults in the checks had emerged, at which time the number and sex of flies in the emergence cages were noted, and the larval medium was examined for delayed larvae or pupae. Later examinations of the larval medium were made if necessary. Four to five days after emergence the flies were offered oviposition medium. A small sample of the eggs was checked for hatching, and a portion of the remainder was placed on untreated larval medium and another generation of flies was reared and observed for abnormalities.

In the screening tests with adult flies, 50 grams of fly food consisting of 6 parts of sugar, 6 parts of powdered nonfat dry milk, and 1 part of powdered egg was treated with 25 ml. of acetone containing 0.05 or 0.5 gram of the chemical in suspension or solution. The food was then allowed to dry for 24 hours, repulverized, and placed in emergence cages containing 100 pupae. Cages containing untreated food were used as checks. Seven days later each cage was examined to note the number of flies emerged, their condition, and any acute toxic effect of the treated food. Two days later another examination was made and oviposition medium was placed in the cage. On the following day the oviposition medium was inspected for eggs. If none were present, the medium was moistened and examined daily until oviposition occurred or all the adults were dead. The viability of any eggs deposited was determined by inspecting the medium for larvae 2 days after oviposition. The larvae were reared to adults and observed for any abnormalities.

RESULTS.—Seventy-nine of the first 200 compounds

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Table 1.—Effect of various chemicals in the larval medium on the development of house flies.[a]

Chemical	Dosage (grams)	Effect on Flies
Acetoacetanilide	0.5	Normal larvae and pupae; no adult emergence
	.1	None
Acetophenetidide	.5	Few larvae and pupae; no adults
	.1	None
Acetophenone	.5	Few larvae, dead before pupation
	.1	None
Acetyl phenylhydrazine	.5	None
	.1	Larvicide
Aldrin	.5	Larvicide
	.1	Few larvae, pupae, adults; no oviposition
Amethopterin	.1	Larvicide
	.05	Normal larvae, 47 pupae dead; of surviving adults 25% wingless, 25% stunted wings, and remainder normal. Mutants isolated, crossed, F_1 normal
	.05	None
	.01	Larvicide
	.01	None
	.005	None
bis(1-Aziridinyl)morpholinophosphine sulfide	.5	Larvicide
	.5	Abnormal pupae, no adults
	.1	Larvicide
	.1	Minute pupae, no adults
	.1	Larvicide
	.01	Larvicide
	.005	None
	.001	None
2,4,6-tris(1-Aziridinyl)-*s*-triazine	.5	Larvicide
	.1	Low adult emergence, no oviposition
	.1	Larvicide
	.05	None
	.01	None
beta-Benzyloxyethyl acrylate	.5	Few adults, normal oviposition and hatch
	.1	None
5-Bromouracil	.5	1 male and 1 female emerged; no oviposition
	.1	Delayed adult emergence (28 days) oviposition light
2,5-bis[*N*,*N*-bis(2-Chloroethyl)-aminomethyl]hydroquinone	0.5	Larvicide
	.1	Larvicide
	.1	Larvae retarded; minute pupae; no adults
	.05	Larvicide
	.01	Larvicide
	.005	None
	.001	None
2,2′′-Dichloro-*N*-methyldiethylamine hydrochloride	.5	Larvicide
	.5	Larvicide
	.1	Larvicide
	.1	None
	.05	Larvicide
	.01	Adults normal; F_1 & F_2 adults minute and retarded in development time
	.005	None
	.001	None
Diphenylacetonitrile	.5	All died in pupal stage
	.1	None
Eugenol	.5	Only 1 female emerged
	.1	None
5-Fluorouracil	0.5	Larvicide
	.1	Larvicide (2 tests)
	.05	Larvicide
	.01	Larvicide
	.005	Larvicide
	.001	None
Indole acetic acid	.5	Larvicide
	.1	Larvicide (2 tests)
	.05	Larvicide
	.01	None
Indole butyric acid	.5	Low adult emergence
	.1	Low adult emergence
l-(-)-Leucine	.5	Low number adults
	.1	None
Methionine	.5	Low adult emergence; no oviposition
	.1	None
bis(1(3)-Methyl-3-acetoxy-propyl)adipate	0.5	None
	.1	Few adults; normal oviposition and hatch
5-Methylhydrouracil	.5	Reduced adult emergence; oviposition normal
	.5	Larvicide
	.1	Larvicide
alpha-(2-Methylphenoxy)-*n*-butyric acid	.5	Larvae small; low adult emergence
	.1	None
alpha-(2-Methylphenoxy)-propionic acid	.5	Larvae and adults stunted
	.1	None
6-Methyluracil	.5	Larvicide
	.1	Larvicide
	.1	None
	.1	Only 1 adult emerged
alpha-Naphthalene acetic acid	.5	Delayed adult emergence
	.1	None
beta-Naphthoxyacetamide	.5	Low adult emergence; no oviposition
	.1	Low adult emergence; no oviposition
	.1	Larvicide
	.05	Larvicide
	.01	None
beta-Naphthoylacetonitrile	.5	F_1 larvae stunted
	.1	F_1 larvae stunted
	.1	Larvicide
	.05	Larvicide
	.01	Low adult emergence
alpha-Naphthyl acetamide	.5	F_1 larvae stunted
	.1	None
Naphthyl methyl isothiourea hydrochloride	0.5	Larvae stunted; no adults
	.1	Larvae and adults stunted; delayed adult emergence
	.1	Larvicide
	.05	Larvicide
	.01	None
alpha-Naphthyl thiourea	.5	F_1 larvae stunted
Nicotinic acid	.5	Larvae small; low adult emergence
	.1	None
alpha-Nitroso-*beta*-naphthol	.5	None
	.1	F_1 larvae small
Orotic acid	.5	Larvicide
	.1	Larvicide
	.1	Few adults; oviposition delayed but normal
6-Pentyl-*m*-tolyl chloroacetate	.5	Larvicide
	.1	Larvicide
	.05	Few minute pupae, 26 days late; minute adults; all dead prior to oviposition age
	.005	None
	.001	None
Piperonyl butoxide	.5	Remained in pupal stage, only 2 adults
	.1	None
Riboflavin	.5	Low adult emergence; little oviposition
	.1	F_1 larvae stunted
Sulfate of 4,5-diaminouracil	0.5	Larvicide
	.1	Larvicide
	.1	Only 1 female and 3 males; no oviposition
Tetramethylene methanesulfonate	.5	One adult emerged; no oviposition
	.1	Low adult emergence; no oviposition
	.1	Larvicide
	.05	Larvicide
	.01	Few pupae; only 3 adults (males) emerged
	.01	None
	.005	None
Thioammelin	.5	Few adults; normal oviposition and hatch
	.1	None

[a] Parent generation = generation exposed to chemical, F_1 = subsequent generation reared on untreated medium.

Table 1.—(Continued)

Chemical	Dosage (grams)	Effect on Flies
Thymine	.5	Only 1 female emerged
	.1	Only 1 female and 3 males emerged; no oviposition
Thymoxyacetic acid	.5	F_1 larvae small
	.1	Delayed and low adult emergence
Triacetin	.5	Minute larvae, few pupae, only 14 adults, normal oviposition and hatch
	.1	None
N,N',N''-Triethylenephosphorothioic triamide	0.5	Larvicide
	.1	Larval period extended 60 days, no adult emergence
	.005	None
	.001	None
Tyrosine	.5	No adults
	.1	Low number adults; no F_1 hatch
	.1	None
	.05	None
	.01	None
Uracil	.5	Larvicide
	.1	Larvicide
	.1	Small adults; no oviposition
Uracil-4-acetic acid	.5	Only 1 adult
	.1	Few adults; no oviposition

tested in the larval medium affected the flies in some stage of development. Acrylonitrile, allethrin, 6-aminouracil, dithiouracil, 6-methyl-2-thiouracil, 5-nitrouracil, 6-propyl-2-thiouracil, 2-thiouracil, and thiourea acted as larvicides at both concentrations; whereas acetanilide, adiponitrile, aldarsone, aldol, allyl sulfide, 9-aminoacridine hydrochloride, amyl acrylate, anethole, aniline fluosilicic acid complex (1:½ mol. ratio), anisaldehyde diethyl acetal, *o*-anisidine, anisonitrile, coumarin, *N,N*-diallylacrylamide, 2,4-dichlorophenoxyacetic acid, dihydrosafrole, 5, 5-dimethyl-2-nonyl-*m*-dioxane, ethyl carbamate, maleic hydrazide, 4-methoxy-1-acetonaphthone, bis(2-methoxyethylmercuri)acetylene, 2-methyl-1,4-naphthoquinone, sulfoxide, and 2,4,5-trichlorophenoxyacetic acid acted simply as larvicides at the higher concentration only. Those that killed all the larvae at 0.1 gram were tested (or will be tested) at lower concentrations for other types of activity. Among the other types of activity observed were stunting of larvae, delayed pupation, failure to pupate, mortality of pupae, delayed adult emergence, sterility, and deformity of pupae and adults. The results with these compounds are given in table 1.

Only 10 of the compounds affected the flies when added to the adult food. The results with these compounds are given in table 2. Some were acute toxicants and others had an effect on the reproductive capacity. The most promising was Amethopterin, also known as Methotrexate®(*N*-[*p*{2,4-diamino-6-pteridylmethyl] methylamine} benzoyl)glutamic acid), which caused sterility at dosages as low as 0.005 gram per 50 grams of food.

Further tests were run to determine the number of days of feeding on amethopterin necessary to inhibit reproduction. Flies were separated as pupae in individual vials, and upon emergence the adults were separated according to sex. In one series of tests some flies were given normal food and others food treated with 0.5% amethopterin for 1, 5, or 30 days, after which all were given untreated food only. Another group of flies were fed untreated food for 5 days and then placed on the treated food for the remainder of their life span. When the flies were 5 days old, in both series of tests, five of each sex were placed together in a cylindrical screen cage. On the sixth day oviposition medium was made available, and the eggs laid were counted and placed in larval medium. Egg viability was determined by inspecting the medium at a later date for

Table 2.—Effect of 10 chemicals on the fertility of house flies.

Chemical	Dosage (grams)	Effect on Flies
Acetyl phenylhydrazine	0.5	Toxicant
	.05	Toxicant
Aldrin	.5	Toxicant
	.05	Toxicant
Allethrin	.5	Toxicant
	.05	None
Amethopterin	.5	No oviposition (adults held for 17 days)
	.125	No oviposition (adults held for 17 days)
	.125	No oviposition (adults held for 17 days)
	.05	No oviposition (adults held for 17 days)
	.05	No oviposition (adults held for 17 days)
	.05	No oviposition (adults held for 17 days)
	.025	No oviposition (adults held for 17 days)
	.025	No oviposition (adults held for 17 days)
	.025	No oviposition (adults held for 17 days)
	.0125	No oviposition (adults held for 17 days)
	.0125	No oviposition (adults held for 17 days)
	.0125	No oviposition (adults held for 17 days)
	.005	No oviposition (adults held for 17 days)
	.005	No oviposition (adults held for 17 days)
	.00125	Oviposition normal, F_1 normal
	.0006	Oviposition normal, F_1 normal
bis(1-Aziridinyl)morpholinophosphine sulfide	.5	Oviposition normal, no hatch
	.5	Oviposition normal, no hatch
	.25	Oviposition normal, F_1 normal
	.125	Oviposition normal, F_1 normal
	.05	Oviposition normal, F_1 normal
	.05	Oviposition normal, F_1 normal
	.05	Oviposition light, no hatch
	.025	Oviposition normal, F_1 normal
	.025	Oviposition normal, no F_1 adults, mortality in larval and pupal stage
2,4,6-tris(1-Aziridinyl)-*s*-triazine	.5	No oviposition
	.5	No oviposition
	.5	Light oviposition, F_1 normal
	.25	No oviposition
	.125	Oviposition normal, no hatch
	.05	Oviposition normal, F_1 normal
	.05	Oviposition normal, F_1 normal
	.05	No oviposition
	.025	Oviposition normal, no hatch
	.025	Oviposition normal, F_1 normal
	.025	Oviposition normal, no hatch
	.0125	Oviposition normal, no hatch
	.0125	Oviposition normal, no hatch
	.005	None
	.0025	None
Benzeneboronic acid	.5	Toxicant
	.05	No oviposition
5-Fluorouracil	0.5	Light oviposition, no hatch
	.5	No oviposition
	.25	Light oviposition, no hatch
	.125	No oviposition
	.05	Oviposition normal, no hatch
	.05	Oviposition normal, no hatch
	.025	Oviposition normal, no hatch
	.025	Oviposition normal, no hatch
	.025	Oviposition normal, no hatch
	.0125	Oviposition normal, F_1 larval life extended for 24 days; no emergence
	.005	None
	.00125	None
Thiourea	.5	Oviposition, no hatch
	.05	None
N,N',N''-Triethylenephosphorothioic triamide	0.5	No oviposition
	.5	No oviposition
	.5	Light oviposition, F_1 normal
	.25	No oviposition
	.125	Light oviposition, F_1 normal
	.05	Oviposition normal, no hatch
	.05	None
	.025	None
	.025	Oviposition normal, no hatch
	.025	Oviposition normal, no hatch
	.0125	Oviposition normal, no hatch
	.0125	Oviposition normal, no hatch
	.005	None
	.0025	None

larvae. Fecundity and fertility were determined by offering oviposition medium in this way at regular intervals until the flies had died or until the end of the 30-day test period. Each test was replicated twice and compared with an untreated check run concurrently. Females initially given treated food, whether for 1, 5, or 30 days, never oviposited; those that were not furnished treated food until the fifth day laid viable eggs on the sixth day but none thereafter, except for one instance when viable eggs were laid on the 14th day.

To determine whether amethopterin sterilized one or both sexes, another series of tests was run in which flies that had fed for 5 days on treated food were crossed with flies of the opposite sex that had fed on either treated or untreated food. Five of each sex were included in a test, and were brought together on the fifth day, after which all flies were fed untreated food for the duration of the testing period. All tests were run in duplicate and compared with untreated checks. Females given treated food for 5 days did not oviposit whether exposed to males fed treated or untreated food, but most females given untreated food oviposited freely when exposed to males fed treated or untreated food, indicating that the sterility caused by amethopterin occurs in the female fly only. These results are similar to those observed by Goldsmith and Frank (1952) in *Drosophila* fed on a related compound, aminopterin.

In another test three flies of each sex were placed in a cylindrical cage upon emergence. They were fed regular food for 5 days, treated food containing 1%, 0.5%, 0.25% or 0.1% of amethopterin on the sixth day and untreated food thereafter, to determine whether the chemical had any sterilant effect if available for only 1 day after a period of normal feeding. Regardless of the concentration, amethopterin had no effect on the initial oviposition but sharply reduced the number of eggs obtained at the second peak of oviposition.

In a larger test 2,000 house fly pupae were placed in each of three rooms, one containing six dishes of food treated with 0.05% amethopterin, another containing three dishes each of treated and untreated food, to permit a choice of diet, and a third containing six dishes of untreated food. On the fifth and ninth days after the flies emerged, oviposition medium was made available for 4 hours. The eggs were allowed to hatch and develop to the pupal stage, and the pupae were counted. In duplicate tests the average number of pupae collected was 100 per test in the rooms where only treated food was available, 458 where a choice of food was available, and 20,065 where only untreated food was present.

Amethopterin would hold much more promise as a practical control agent if it sterilized the male flies as well as the females, but even so it appeared to merit further consideration.

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New Techniques for Screening Cockroach Repellents[1]

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Abstract

Two new methods for screening repellents for cockroaches are described. The slanting card method is based on the tendency of cockroaches to climb a slanting surface. Cards impregnated with test chemicals are placed in a slanting position against the inside walls of a transparent plastic box containing a colony of cockroaches. The number of insects on the treated cards is compared with the number on an untreated card. The ground glass cylinder method employs three intercommunicating glass cylinders ground on the inside to keep the cockroaches on treated filter papers or other surfaces at the bottom. By this method the best repellents from more than 1000 chemicals tested were 2,3,4,5-bis(Δ^2-butenylene)tetrahydrofurfural (R-11), 2-hydroxyethyl *n*-octyl sulfide (R-874), 2-hydroxypropyl *n*-octyl sulfide (R-949) and *tert*-butylsulfenyl dimethyl dithiocarbamate (R-55). (N(2-Ethylhexyl)bicyclo-2,2,1-hept-5-ene-2,3-dicarboxamide (MGK-264) is synergistic with R-11. Although highly effective, R-55 is more toxic and has more odor than R-11 or R-874. R-874 appears superior to R-11 as a cockroach repellent.

The use of repellents in the control of cockroaches is of interest where resistance to insecticides has become a problem and where repelling the insects is more desirable than killing them. Some logical places to use repellents could be in paperboard cartons for food, soft drink and

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beer; around food where highly toxic insecticides cannot be used; and in juke boxes or other coin operated machines. They may be used in some cases in combination with insecticides.

Screening chemicals as cockroach repellents is underway in several laboratories. Most of the methods are based on the rejection by the insects of some type of treated shelter such as ice cream cartons proposed by Goodhue & Tissol (1952). Burden & Eastin (1960) used a similar method. Hazard (1945) tested the repellency of various magnesium oxychloride cements by providing a choice of surfaces. Block (1951) used small pieces of book binding and food in a filter paper envelope both of which were treated with various repellents.

Most methods measure the effectiveness of a repellent by its ability to prevent insects from feeding or from seeking shelter from light. The methods described in this paper are based on allowing the cockroaches to choose between untreated and chemically treated surfaces. Both are suitable for screening large numbers of chemicals. One method is based on the tendency of cockroaches to crawl up a slanting surface and works well with the German cockroach. The other method employs glass cylinders with communicating passages and sand-blasted walls to retain the cockroaches and is better suited for the larger American and Oriental cockroaches.

CHEMICALS TESTED.—The chemicals used as examples in these tests for the most part have long names so they are listed below with their numbers. The letter "R" is prefixed to the numbers of chemicals having good repellent action, but the numbers themselves have no significance except for identification. These numbers will be used in the remainder of the paper.

R-11[2]	2,3,4,5-Bis(Δ^2-butenylene)tetrahydrofurfural
R-55[3]	*tert*-Butylsulfenyl dimethyl dithiocarbamate
R-874[3]	2-Hydroxyethyl *n*-octyl sulfide
R-949[3]	2-Hydroxypropyl *n*-octyl sulfide
1131[4]	Di-*n*-butyl succinate
MGK-264[#2]	N(2-Ethylhexyl)bicyclo-2,2,1-hept-5-ene-2,3-dicarboxamide

R-11 and 1131 are commercially available. R-55, R-874 and R-949 are in the development stage. MGK-264 is a commercially available pyrethrum synergist.

SLANTING CARD METHOD[5].—A colony of German cockroaches (*Blattella germanica* (L.)) was maintained in a transparent plastic box measuring 8″ x 12″ on the bottom and 8″ high. The top was closed by a flanged cover holding 60-mesh brass screen (see fig. 1). They were fed dog-food cubes and watered in 1-inch test tubes lying in a horizontal position with cotton plugs to retain the water. Debris was allowed to accumulate and each of the colonies appeared to reach a maximum of about 2000 cockroaches of all ages. The cages were always exposed to the ordinary fluorescent lights of the laboratory which did not disturb the insects. The upper 1 inch of the inside wall was greased with petroleum jelly. However, some young cockroaches crawled out where the lid did not fit perfectly. A strip of adhesive tape around the outside of the lid prevented them from crawling out.

File cards, size 3″ x 5″, provided a good test surface. Other materials used were sections of a beer carton, fiber board, sheet metal, glass, et cetera. The file cards were dipped once in acetone solutions containing 5 gm. per 100 ml. of the chemicals and hung up to dry overnight. The amount of chemical deposited, assuming all is on the surface, was determined to be 0.2175 mg. per sq. cm. by measuring the amount of solution required to wet a card. Some was absorbed by the fiber so there was actually less on the surface. If this amount was highly repellent the chemical was retested at a lower dosage.

Table 1.—Typical repellent test against German cockroaches by the slanting-card method showing numbers on cards and per cent repellency after three intervals.

Category	Interval in Hours	Cards Having 0.2175 Mg./Sq. Cm. of Repellent				
		R-11	1131	R-55	R-874	Check
No. of cockroaches on each card at hourly intervals	1	43	208	2	0	215
	2	40	208	6	0	209
	3	41	166	2	0	205
	4	26	164	4	0	201
	5	29	176	2	0	187
	6	29	198	7	0	232
Total number	2	83	416	8	0	424
Repellency (%)		81	2	98	100	
Total number	4	150	746	14	0	830
Repellency (%)		82	10	98	100	
Total number	6	208	1,120	23	0	1,249
Repellency (%)		83	10	98	100	

The next day the plastic cages containing the cockroaches were placed on a uniformly lighted work table where all sides could be observed. At 9:00 a.m. the cards were placed with the longest dimension at an angle of about 25 degrees against the inside wall of the plastic box. One untreated check and four treated cards were generally used. Enough cockroaches were present to saturate all the cards on both sides. Larger cards could be used if the total area does not exceed 150 sq. in.

The cockroaches crawled up the untreated card immediately and tested the others. They soon learned to avoid the cards which contained an effective repellent. The number found on the treated cards at the end of 1 hour was an indication of the repellency. Figure 1 shows different views of a test in progress.

The complete data recorded from a typical run are shown in table 1. The per cent repellency based on the untreated check was calculated at the end of 2, 4, and 6, hours. A comparison of the results after these intervals indicated that four hourly readings are enough and two could be used without exceeding the probable biological variation expected by such a method.

With less than a full colony of cockroaches the population pressure was less and the results were sometimes different. Table 2 gives the results of a comparison of the percentage repellency for these same materials at three dosages where 100, 500 and a full colony of cockroaches were used. It was obvious during the test, and from the results, that 100 cockroaches were not enough. The results were more reliable with 500 cockroaches but a chem-

[2] McLaughlin Gormley King Company, Minneapolis, Minn.
[3] Phillips Petroleum Company, Bartlesville, Okla.
[4] Glenn Chemical Company, Chicago, Ill.
[5] The author wishes to acknowledge the suggestion of this method by K. E. Cantrel and his assistance in the refinement of both the methods described in this paper.

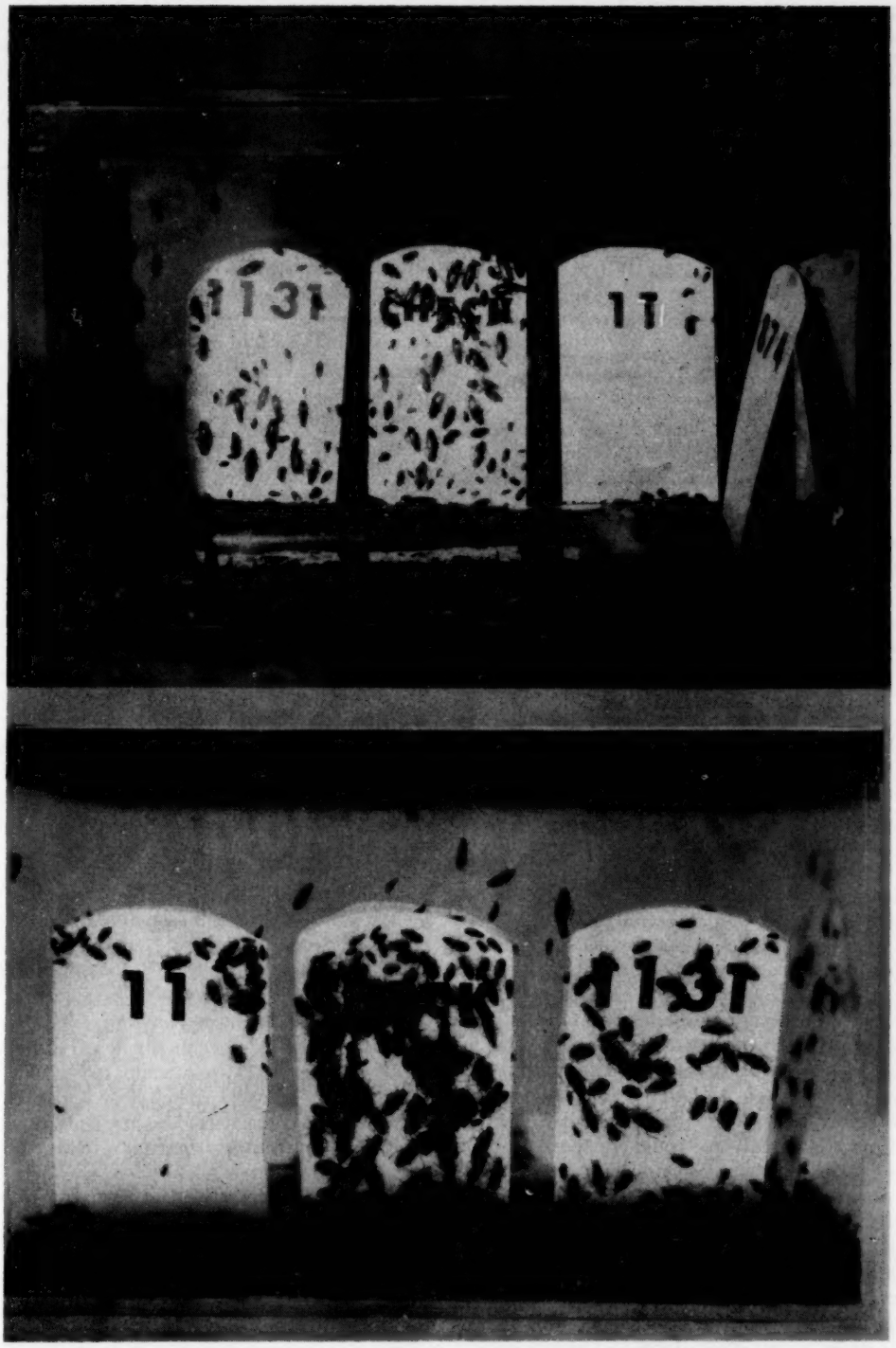

FIG. 1.—Repellent test by slanting card method, upper photograph shows transparent plastic box and front view of cockroaches on treated and untreated cards. Lower photograph shows the backs of the same cards.

ical sometimes appeared more repellent than with the full colony. The results were the most consistent and reliable with at least 2000 cockroaches present.

In another variation, the cockroaches were shaken off the cards after each reading. This increased the traffic over the repellent treated surfaces and reduced the repellency slightly. The cockroaches moved about by themselves so that this was unnecessary, especially if only two readings were taken. Aluminum powder was placed on one card as an indicator. Nearly every cockroach in the colony had the powder on the tarsa which showed the extent of the activity.

Table 2.—Comparison of four repellents to German cockroaches using three dosages at three population levels by the slanting-card method.

Repellent	No. of Cockroaches Used	Per Cent Repellency at Three Dosages in Mg./Sq. Cm.		
		0.0435	0.087	0.2175
R-11	100	Negative	Negative	100
1131	100	Negative	Negative	Negative
R-55	100	100	100	100
R-874	100	100	100	100
R-11	500	Negative	44	99
1131	500	31	Negative	43
R-55	500	100	96	99
R-874	500	90	97	100
R-11	2,000+	Negative	11	83
1131	2,000+	Negative	5	10
R-55	2,000+	92	98	98
R-874	2,000+	23	98	100

Adult American cockroaches (*Periplaneta americana* (L.)) were tried but they are more nervous and a slight disturbance excited the whole colony. The immature stages might be more adaptable but these were not tried. Oriental cockroaches (*Blatta orientalis* L.) have not yet been used in testing this method.

This is a rapid, useful method. It is not the ultimate and is, no doubt, subject to refinements. The repellency of a chemical as determined in this way is affected to some extent by the other chemicals being tested at the same time and is, therefore, a comparative rather than an absolute method. It is not adaptable to mixtures of repellents and insecticides without sacrificing the colony. Even with its limitations the method has proved satisfactory for studies of cockroach repellents for the past several years in this laboratory.

GROUND GLASS CYLINDER METHOD.—The apparatus used in this method is most easily described by referring to figure 2. It was made from three 4″ x 4″ glass cylinders with U-shaped openings at the bottom to allow free passage of the cockroaches between the cylinders. The inside of each cylinder was sand blasted and ground further with medium emery cloth to produce a glass powder coating. This prevented most adult cockroaches from climbing out but a few small ones could crawl over it. The three cylinders with openings coinciding were taped together and placed on a piece of window glass to form a bottom. If the connecting archways did not fit tightly the leaks were covered with adhesive tape. Eight sets were used simultaneously.

Filter papers were slipped under each cylinder and five American cockroaches were introduced the evening before a test was to start to give them time to become accustomed to their new surroundings and to learn the passageways from one cylinder to another. This type of enclosure is well suited for adults or large nymphs of the American cockroach and for similar Oriental cockroaches. Tests were also made on German cockroaches using 10 adults but they were more difficult to retain.

The day previous to testing, 11-cm. Whatman No. 40 filter papers were dipped in acetone solutions containing 5 grams per 100 ml. of the test chemical to give 0.35 mg. per sq. cm. if all remained on the two surfaces. The amount was somewhat less since not all of the chemical remained on the surface.

The next day at 9:00 a.m. the dried, treated papers were substituted for the two untreated papers under the outer cylinders and a clean paper was placed under the center one. The treated papers carried the code number of the chemical and the date. The cockroaches on each treated paper and on the center were counted at the end of an hour. The cockroaches were stirred to force them to again choose the least objectionable paper. Stirring appears necessary to prevent stagnation. The reading and stirring process was repeated five times at hourly intervals the first day and on the second day at 9:00 and 3:00. It is believed this 2-day period is long enough to obtain an indication of the repellency of a chemical. The cockroaches were watered after the first test and used once more.

The results of a typical test in which some chemicals were compared with R-11 are shown in table 3. Except for 1131, *n*-butyl succinate, these are the best ones selected from more than 1,000 chemicals. From the preliminary result the best repellents were selected and tested again against each other until only the very best ones remained. Freshly dipped papers, prepared as above directed, are best for such comparisons. The odor of cockroaches on used papers can be attractive and interfere with the test. Aging tests were often made on the best repellents. A number of filter papers were impregnated with the repellent to be tested and hung in the laboratory to age. One of each was tested at certain intervals and discarded. This method will also indicate residual insecticidal activity. Toxicity was observed with some of the chemicals.

This method is adaptable for tests on various surfaces. A typical test is shown in table 4. Squares of about $4\frac{1}{4}$ inches were cut from glass, painted wood, linoleum and masonite. Some of the repellents gave better results on one surface than on another. The results did not appear to be related to the porosity of the surface.

The test is well adapted for comparative studies. Table 5 shows a series of tests in which varying proportions of MGK-264 and R-11 were compared with R-11 alone on three species of cockroaches over a period of 2 weeks. These results show that the mixture is more effective after aging than R-11 alone.

Table 3.—A typical repellent test using American cockroaches in the ground glass cylinder method.

Repellent No.	Position of Cockroaches at Indicated Time in Hours								Total of 8 Counts	Per Cent Repellency
	1	2	3	4	5	6	24	30		
R-874	2	1	0	0	0	0	0	0	3	93
Untreated center	2	3	3	3	3	2	5	4	25	
R-11	1	1	2	2	2	3	0	1	12	70
949	0	0	0	0	0	0	4	0	4	90
Untreated center	5	5	0	2	5	4	1	5	27	
R-11	0	0	5	3	0	1	0	0	9	78
55	0	0	0	0	0	1	0	0	1	97
Untreated center	5	4	4	5	5	4	4	5	37	
R-11	0	1	1	0	0	0	1	0	3	93
1131	2	2	3	2	3	3	4	3	22	45
Untreated center	3	3	2	3	2	2	1	2	18	
R-11	0	0	0	0	0	0	0	0	0	100

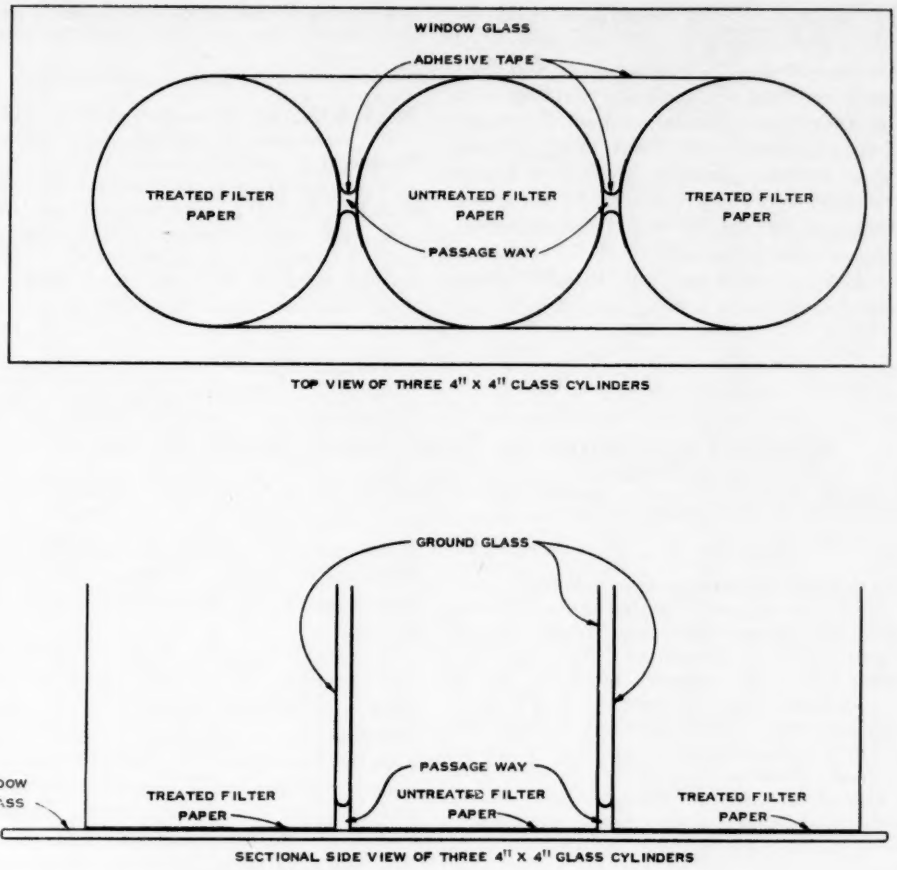

FIG. 2.—Diagram of cockroach repellent test apparatus.

A variation of the method was tried in which only two cylinders were used. Repellent treated papers under each should force the cockroaches to choose the least objectionable one. However, the results were less consistent than they were with an untreated paper in the center of a three-cylinder apparatus. The cockroaches apparently became desensitized and confused.

Several possible variations may be made in this method. The number of cockroaches, the number of readings, and the time interval may all be varied. The test might be shortened to 1 day or extended for more than 2 days. The light intensity is a factor. Perhaps the cockroaches could be fed and watered in these cylinders and used for more tests.

Table 4.—Comparison of R-11 with R-55 and R-949 on four different surfaces as repellents to American cockroaches by the glass cylinder method.

Repellent No.	Days Aging Time	Per Cent Repellency on Different Surfaces			
		Painted Wood	Glass	Linoleum	Masonite
R-55	1	97	100	93	100
R-11		80	90	97	100
R-55	7	97	100	75	95
R-11		22	47	80	35
R-55	14	90	87	80	95
R-11		97	90	90	87
R-949	7	35	65	87	52
R-11		87	72	90	97
R-949	14	97	70	77	100
R-11		70	87	87	97

Table 5.—Extended effect of MGK-264 with R-11 determined by the glass cylinder method.

Repellents and Mixtures at 0.355 Mg./Sq. Cm.	Per Cent Repellency to Three Species of Cockroaches after 1, 7 and 14 Days								
	German			American			Oriental		
	German Cockroaches after 1, 7 and 14 Days			American Cockroaches after 1, 7 and 14 Days			Oriental Cockroaches after 1, 7 and 14 Days		
	1	7	14	1	7	14	1	7	14
R-11, 1 to 1 ratio with MGK-264[a]	100	97	100	100	57	95	90	97	100
R-11	100	90	94	100	97	20	100	22	45
R-11, 2 to 3 ratio with MGK-264	97	100	90	100	70	78	100	95	100
R-11	100	19	87	97	87	66	100	25	28
R-11, 1 to 4 ratio with MGK-264	97	99	99	87	95	72	100	92	97
R-11	100	90	58	97	78	40	95	78	22

[a] MGK has no significant repellency to cockroaches alone.

Although this paper is devoted primarily to a discussion of methods, a few comments on the repellents used as examples are presented. R-55 appears to be the most intense cockroach repellent by both the slanting card method and the ground glass cylinder method. This repellent was first found by Goodhue & Tissol (1952) by using treated ice cream cartons. Recently Burden & Eastin (1960) found it gave over 50% repellency in ice cream cartons for 91 days. R-55 is highly effective, but is more toxic and has more odor than R-11 or R-874. R-874 is suitable for use as a cockroach repellent. R-949 is a similar compound and is similar in activity to R-874. MGK-264 increases the initial effectiveness and durability of R-11.

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Spider Populations in New Jersey Apple Orchards[1]

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ABSTRACT

Mean numbers of spiders in three unsprayed New Jersey apple orchards were higher than in seven sprayed orchards during 1957. "Hunting" spiders appeared to be more strongly affected by sprays than web-builders. Spiders formed a lower proportion of total predatory arthropods (excluding mites) in sprayed orchards than in unsprayed. A seasonal trend was noted in population density similar to that observed elsewhere. Eight species comprised about 87% of the individuals from July to October in unsprayed orchards. These species made up only 60% of the individuals in sprayed orchards, the difference being mainly owing to the relative abundance of the erigonid, *Grammonota inornata* Emerton. The 34 species found in the study are listed with annotations.

The role of insects as natural enemies of apple orchard pests has been studied for many years, but the importance of orchard spiders has usually been neglected. Exceptions are the recent studies reported by Chant (1956), Dondale (1956, 1958), and LeRoux (1959). As Gertsch (1949, p. 236) points out, spiders are "among the dominant predators of any terrestrial community." At the present time, however, we cannot generalize on the influence of spiders on pest populations, except to state that they feed mainly on any suitable prey that is present in abundance, irrespective of whether the prey is a plant-feeder or another predator. The fact that they sometimes feed on beneficial organisms has been considered by some workers (*e.g.*, Kaston 1948, p. 40) to reduce their value to man as controlling agents. On the other hand, this property may be an asset by which they are enabled to survive periods of low pest density.

Before an evaluation of orchard spiders can be made, there must be considerable information on their kinds and numbers in the environment, and on their biology and behavior. The papers already cited are a beginning toward this goal, and this paper presents additional data from a group of New Jersey orchards. The senior author, while engaged in a study of the effects of spray programs on orchard insect and mite populations in that state (Specht 1959, Thomas *et al.* 1959) collected predatory arthropods in several orchards in 1957. The spiders in these collections, because of their universal occurrence and frequent abundance, are considered first. They were identified by the second author, and are deposited in the collections of the Research Station in Kentville, Nova Scotia.

METHODS.—The 10 study orchards were in various parts of north, central, and south New Jersey. Seven of these were under a "modified" spray program, *i.e.*, the phosphates and chlorinated hydrocarbons previously used by the owners were, from the beginning of the 1957 season, replaced by the less potent materials, ryania, lead arsenate, and nicotine bentonite. These orchards were compared with three that were in a state of partial neglect, receiving fertilizer but no sprays.

The predators, including spiders, were jarred from trees with a stick onto a yard-square, cloth-bottomed tray, from which they were taken with an aspirator. The aspirator contained a small amount of 70% ethyl alcohol to inactivate them. Each sample consisted of predators taken from four trees forming a square. Only the lower branches of the inner quadrant of each of the four trees were jarred to obtain a sample.

The spiders were identified with the help of Kaston's (1948) monograph, Archer's (1940, 1941) papers on Argiopidae, and recent revisions of the genera *Theridion* (Levi 1957) and *Philodromus* (Dondale 1959).

RESULTS AND DISCUSSION.—Among the differences noted between sprayed and unsprayed orchards was the greater density of spiders in the latter (tables 1, 2). The difference was not as pronounced as that reported in England, however, where Chant (1956) found that the regular spray schedules, which involve such materials as caustic winter washes, lime sulphur, and parathion, reduced spider populations to small numbers of web-builders. Materials used in the New Jersey sprayed orchards apparently permitted much of the normal spider fauna to sur-

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Table 1.—Mean numbers of spiders per sample in three unsprayed New Jersey apple orchards in 1957.

FAMILY	JULY		AUGUST	SEPTEMBER		OCTOBER	
	1–15	16–31	1–15	1–15	16–30	1–15	16–31
Number of samples	1	1	2	4	2	2	4
Theridiidae	0	23	7.5	9.0	11.0	14.0	5.5
Erigonidae	0	0	0	0.3	0	0	0.5
Argiopidae	2	4	2.5	1.7	2.0	3.0	2.8
Other web-builders	0	2	0.5	0.3	0.5	0.5	1.0
Anyphaenidae	2	24	9.5	8.2	2.0	5.0	2.2
Thomisidae	5	25	12.0	9.7	6.5	6.5	7.3
Salticidae	1	26	10.5	9.7	8.5	11.0	4.2
Other nonweb-builders	0	0	3.0	0.7	1.0	0	1.3
Total spiders	10	104	45.5	39.6	31.5	40.0	24.8
Total predators (except mites)	54	213	47.0	45.5	72.0	46.0	31.5
Spiders as percentage of total predators	18.5	48.8	95.7	87.0	43.9	87.0	78.7

Table 2.—Mean numbers of spiders per sample in seven New Jersey apple orchards under a modified[a] spray program in 1957.

FAMILY	MAY	JUNE	JULY		AUGUST		SEPTEMBER		OCTOBER		NOVEMBER
	16–31	16–30	1–15	16–31	1–15	16–31	1–15	16–30	1–15	16–31	1–15
Number of samples	4	1	4	4	7	9	17	8	11	18	4
Theridiidae	1.3	0	0	1.2	5.0	5.6	5.8	7.6	8.1	5.1	1.4
Erigonidae	0	0	0	0.2	0	0.1	0.2	1.7	3.3	8.9	0.3
Argiopidae	0.5	0	0.2	0.9	0.2	2.3	1.2	1.7	0.6	1.7	0
Other web-builders	0	0	0	0	0	0.4	0.1	0.2	0.4	0.9	0.5
Anyphaenidae	0	6	2.1	1.9	1.2	1.5	1.0	2.0	2.2	1.4	0
Thomisidae	0.2	1	1.3	2.4	4.3	2.5	3.4	2.1	4.8	3.5	0.4
Salticidae	0	1	0.2	1.2	2.0	3.3	3.0	5.0	3.9	3.0	0.3
Other nonweb-builders	0	0	0.2	1.4	1.0	0.3	0.3	0.4	1.4	0.8	0.6
Total spiders	2.0±0.4[b]	8	4.0±0.9	9.2±2.6	13.7±1.2	16.1±3.7	15.0±2.3	20.7±3.7	24.7±4.0	25.3±3.8	3.5±0.5
Total predators (except mites)	7.0	14	25.8	118.3	110.4	51.3	40.6	47.2	50.0	38.6	7.5
Spiders as percentage of total predators	28.6	57.1	15.5	7.8	12.4	31.4	37.0	43.9	49.4	65.5	46.7

[a] Insecticides: ryania, lead arsenate, nicotine bentonite.
[b] Standard error.

vive; densities in these orchards were, in fact, quite similar to those in Nova Scotia, where "modified" spray programs (Pickett & Patterson 1953) are used by the majority of growers. In the unsprayed New Jersey orchards the largest single component of the spider population consisted of tactually-dependent "hunters" (table 3). The reduction of both visually-dependent and tactually-dependent "hunters" in sprayed orchards may have been owing to the greater effect of sprays on these spiders, which move about and take their prey on the surfaces of branches and foliage.

Another difference was the higher proportion of spiders to total predators (exclusive of predatory mites) in unsprayed orchards. In all but two of the half-monthly sampling periods, spiders exceeded 50% of total predators, the highest percentage being 95.7 (table 1). In the group of sprayed orchards, the proportion of spiders fell short of 50% during most sampling periods (table 2). There were relatively large populations of phytophagous mites in these orchards during late July and early August, which favored the presence of large numbers of Coccinellidae and Chrysopidae (Specht 1959, Thomas *et al.* 1959), thus reducing the percentage of spiders.

The seasonal trend in populations inhabiting sprayed orchards was approximately the same as that reported for Nova Scotia, where Dondale (1958) observed a small peak in the spring and a larger one in late summer or early autumn. In the data from unsprayed orchards, however, total spiders appeared to fluctuate without showing any distinct trend from July to October. There is no ready explanation for this, although the trend may simply be obscured by the variability between samples, the number of samples being low.

Table 4 shows the relative status of each of the most abundant species in the population, based on total individuals during the period July to October. In unsprayed orchards, a group of eight species constituted about 87% of the total individuals. The web-builder *Theridion murarium* Emerton and the "hunting" spiders *Anyphaena celer* (Hentz), *Philodromus vulgaris* (Hentz), and *Metaphidippus galathea* (Walckenaer) were particularly abun-

Table 3.—Composition of spider populations by ecotaxonomic groups in sampling New Jersey apple orchards, July to October, 1957.

CATEGORY	UNSPRAYED ORCHARDS		SPRAYED ORCHARDS	
	No. Individuals per Sample[a]	Per Cent	No. Individuals per Sample[a]	Per Cent
Web-builders	13.4	32	7.8	51
Visually-dependent "hunters" (Salticidae)	10.1	24	2.2	14
Tactually-dependent "hunters" (Thomisidae, Anyphaenidae, etc.)	18.7	44	5.3	35
Total	42.2	100	15.3	100

[a] Spiders jarred from the lower branches of the equivalent of one tree.

Table 4.—Numerical status of various species of spiders sampled in New Jersey apple orchards, July to October, 1957.

Species	Unsprayed Orchards		Sprayed Orchards	
	No. per Sample[a]	Per Cent of Total	No. per Sample[a]	Per Cent of Total
Theridion murarium	7.3	21	3.3	19
Anyphaena celer	5.9	17	1.2	7
Metaphidippus galathea	4.7	13	1.3	7
Philodromus vulgaris	4.1	12	1.1	7
Hentzia palmarum	2.9	8	0.4	2
Misumenops asperatus	2.6	7	1.9	10
Philodromus rufus	1.8	5	0.3	2
Araniella displicata	1.4	4	0.8	4
Others	4.4	13	7.3	42
Total	32.5	100	18.3	100

[a] Spiders jarred from the lower branches of the equivalent of one tree.

dant, these four species together forming about 63% of the individuals. The average density of the following species appeared to be higher in these orchards:

Theridion murarium Emerton
Anyphaena celer (Hentz)
Philodromus vulgaris (Hentz)
Metaphidippus galathea (Walckenaer)
Hentzia palmarum (Hentz)

Species that occurred at only slightly higher densities in unsprayed orchards were:

Araniella displicata (Hentz)
Misumenops asperatus (Hentz)
Philodromus rufus Walckenaer

The density of the species indicated as "others" in table 4 was higher in sprayed orchards than in unsprayed. The percentage of total individuals represented by these species was also greater in the sprayed orchards. Nearly half of this increase was attributable to a single species of Erigonidae, namely, *Grammonota inornata* Emerton, which appeared in two of the sprayed orchards in large numbers during October. Also contributing significantly to the increase were *Theridion albidum* Banks, *Oxyopes salticus* Hentz, and *Paraphidippus marginatus* (Walckenaer), which were found in most orchards throughout the study period.

Thirty-four species were found in the study, nine of which were unidentified at the species level because they were represented only by sexually immature specimens. The 34 species were distributed among 28 genera in 13 families. Slightly less than half of the species were web-builders, the remainder being visually-dependent "hunters" (Salticidae, 18%) or tactually-dependent "hunters" (Thomisidae, Anyphaenidae, Clubionidae, Oxyopidae, and Lycosidae, 36%). Thomisidae alone comprised about 18% of the total. The numbers of species occurring in the three main groups, with comparable figures from other areas, are shown in table 5.

LIST OF SPECIES COLLECTED.—Brief statements of ecological or taxonomic significance are given for most species. Unless otherwise indicated, they were found in both sprayed and unsprayed orchards. The term "common" is applied to species found in all or most of the orchards examined; "abundant" refers to relatively dense populations in one or more orchards. If the species was already known from New Jersey, a literature reference is given.

THERIDIIDAE.—*Enoplognatha* sp.—One immature specimen from an unsprayed orchard at Haddonfield, Camden County. Spiders of this genus normally live near the ground (Levi 1957).

Theridion albidum Banks.—Common, not abundant. Collected in vicinity of New Brunswick, Middlesex County; vicinity of Oxford, Warren County; and at Haddonfield. Reported from Bergen County, New Jersey, by Levi (1957). Also reported from apple orchards in Nova Scotia (Dondale 1956).

Theridion differens Emerton.—Common, not abundant. Collected in vicinities of New Brunswick and Oxford, and at Haddonfield. Reported from Bergen, Burlington, and Ocean Counties, New Jersey by Levi (1957). Also reported from apple orchards in Nova Scotia (Dondale 1956) and Quebec (LeRoux 1959).

Theridion murarium Emerton.—Common, also the most abundant species in both types of orchard. Collected in vicinities of New Brunswick and Oxford, and at Haddonfield. Reported from Bergen, Passaic, and Ocean Counties, New Jersey by Levi (1957). Also reported from Maryland (Muma 1945), Connecticut (Kaston 1948), and from apple orchards in Nova Scotia (Dondale 1956) and in Quebec (LeRoux 1959).

Theridion rabuni Chamberlin and Ivie.—One female from an unsprayed orchard in vicinity of New Brunswick. Reported from Gloucester County, New Jersey, and from fields in Maryland by Levi (1957).

LINYPHIIDAE.—*Frontinella communis* (Hentz).—Two specimens from sprayed orchards in late October and November, in vicinity of New Brunswick and at Haddonfield. Reported from grassy fields in Morris County, New Jersey by Rapp & Rapp (1946).

ERIGONIDAE.—*Ceraticelus fissiceps* (O. P.-Cambridge).—One specimen from a sprayed orchard in vicinity of New Brunswick.

Eridantes erigonoides (Emerton).—One specimen from an unsprayed orchard in vicinity of Oxford. Reported by Muma (1945) from a variety of habitats in Maryland including houses, grass, and under boards.

Grammonota inornata Emerton.—Common, occasionally abundant in late summer in sprayed orchards. Collected in vicinities of New Brunswick and Oxford, and at Haddonfield. Reported heretofore from houses in Maryland (Muma 1945), and from ground habitats, particularly on open beaches (Kaston 1948).

Table 5.—Comparison of numbers of species of spiders from orchards in four apple-growing regions of North America and Europe.

Category	New Jersey[a]	England[b]	Nova Scotia[c]	Quebec[d]
Web-builders	16	39	48	7
Visually-dependent "hunters"	6	1	10	4
Tactually-dependent "hunters"	12	12	19	10
Total	34	52	77	21

[a] One season's collecting.
[b] Three season's collecting (Chant 1956).
[c] Four season's collecting (Dondale 1956).
[d] Sporadic collecting (LeRoux 1959).

ARGIOPIDAE.—*Eustala anastera* (Walckenaer).—Common, not abundant. Collected in vicinities of New Brunswick and Oxford, and at Haddonfield. Kaston (1948) reported this species as being abundant in many localities in Connecticut.

Mangora placida (Hentz).—One specimen from a sprayed orchard at Haddonfield in early November.

Neoscona domiciliorum (Hentz).—Fairly common, never abundant. Found in sprayed orchards in vicinities of New Brunswick and Oxford. A southern species with "a rather scattering distribution south of the fall line" (Archer 1940, p. 49).

Araniella displicata (Hentz).—Common and abundant in north New Jersey orchards. Collected in vicinities of New Brunswick and Oxford, and at Haddonfield. Abundant also in Connecticut (Kaston 1948), and in orchards in Nova Scotia (Dondale 1956, 1958) and Quebec (LeRoux 1959). Apparently uncommon south of New Jersey (Archer 1940, Muma 1945).

Tetragnathidae.—Tetragnatha sp.—Common, not abundant. Immature specimens found in vicinities of New Brunswick and Oxford, and at Haddonfield.

MIMETIDAE.—*Mimetus epeiroides* Emerton.—Four specimens from sprayed orchards in vicinity of New Brunswick and at Haddonfield. Members of this genus considered to feed exclusively on other spiders, particularly argiopids and theridiids (Gertsch 1949, p. 174).

LYCOSIDAE.—*Pirata* sp.—Four immature specimens from vicinities of New Brunswick and Oxford. These spiders normally live on the ground.

OXYOPIDAE.—*Oxyopes salticus* Hentz.—Common, but abundant only in vicinity of New Brunswick and at Haddonfield. Also known from many localities in Maryland (Muma 1945), but relatively rare in Connecticut (Kaston 1948).

Oxyopes scalaris Hentz.—Four specimens from sprayed orchards in vicinity of New Brunswick and at Haddonfield. Reported by Kaston (1948) to be more common than the foregoing species in Connecticut.

CLUBIONIDAE.—*Clubiona* sp.—Three immature specimens from a sprayed orchard at Haddonfield.

Trachelas tranquillus (Hentz).—Two females, one immature specimen from sprayed orchards in vicinity of New Brunswick and at Haddonfield. Reported previously from houses and under boards and stones (Muma 1945, Kaston 1948).

ANYPHAENIDAE.—*Anyphaena celer* (Hentz).—Common, also the second most abundant species in unsprayed orchards. Less abundant in sprayed orchards. Collected in vicinities of New Brunswick and Oxford, and at Haddonfield. Reported by Kaston (1948) to be the commonest of four species of *Anyphaena* in Connecticut, where it is found on the ground as well as in low shrubs.

THOMISIDAE.—*Misumenops asperatus* (Hentz).—Common and abundant. Collected in vicinities of New Brunswick and Oxford, and at Haddonfield. Recorded from Morris County, New Jersey (Rapp & Rapp 1946) and reported as being "extremely common" in Connecticut (Kaston 1948). One of the dominant species in Quebec orchards (LeRoux 1959).

Tmarus sp.—One immature specimen from a sprayed orchard at Haddonfield.

Coriarachne sp.—One immature specimen from a sprayed orchard in vicinity of Oxford.

Xysticus sp.—Four immature specimens from vicinity of New Brunswick and at Haddonfield.

Philodromus rufus Walckenaer.—Common and abundant, but less abundant at Haddonfield than in vicinities of New Brunswick and Oxford. "Found in abundance in sweeping bushes and grass" in Connecticut (Kaston 1948); one of the principal orchard spiders in Nova Scotia (Dondale 1956, 1958) and in Quebec (LeRoux 1959).

Philodromus vulgaris (Hentz).—Common and abundant. Collected in vicinities of New Brunswick and Oxford, and at Haddonfield. Recorded from Ramsey, New Jersey by Dondale (1959). Also reported as being "extremely common" in Connecticut (Kaston 1948) and found occasionally on apple tree trunks in Nova Scotia (Dondale, unpublished) and in Quebec (LeRoux 1959). This species was synonymized with *P. pernix* Blackwall by Banks (1893, p. 125), but a study of the original descriptions and type localities of these species indicates that they are distinct, and that the name *vulgaris* should be reinstated (Dondale 1959).

SALTICIDAE.—*Sitticus* sp.—Two immature specimens from a sprayed orchard at Haddonfield.

Metaphidippus galathea (Walckenaer).—Common and abundant, particularly abundant in vicinity of New Brunswick and at Haddonfield, less so in vicinity of Oxford. Reported from Morris County, New Jersey by Rapp & Rapp (1946). Considered by Kaston (1948) to be "more common" in the southern states than in the north; replaced in the north by *M. protervus* (Walckenaer). Rare in Nova Scotia orchards (Dondale 1956).

Paraphidippus marginatus (Walckenaer).—Common and fairly abundant, but less abundant at Haddonfield than in vicinities of New Brunswick and Oxford. "Extremely common" in Connecticut (Kaston 1948). One of the principal species in apple orchards in Nova Scotia (Dondale 1956, 1958), and in Quebec (LeRoux 1959).

Phidippus audax (Hentz).—Common, but not abundant. Collected in vicinities of New Brunswick and Oxford, and at Haddonfield. Very common also in Maryland (Muma 1945) and Connecticut (Kaston 1948). Reported recently as a predator of the basilica spider, *Allepeira lemniscata* Walckenaer, in Maryland (Lamore 1958).

Phidippus hirsutus Barrows.—One male, several immature specimens from two sprayed orchards in vicinity of New Brunswick and at Haddonfield. Considered a rare species, known in the mature state only from the male sex (Kaston 1948).

Hentzia palmarum (Hentz).—Common and abundant. Found in vicinities of New Brunswick and Oxford, and at Haddonfield. Known also from Maryland (Muma 1945) and Connecticut (Kaston 1948).

DICTYNIDAE.—*Dictyna* sp.—Not common or abundant. Found in vicinities of New Brunswick and Oxford, and at Haddonfield.

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Effectiveness of Ruelene Applied as Localized "Pour-On" and as Spray for Cattle Grub Control[1]

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ABSTRACT

Ruelene® (4-*tert*-butyl-2-chlorophenyl methyl methylphosphoramidate), formulated as a 4 lb./gal. emulsifiable concentrate, was applied to cattle for the control of cattle grubs, *Hypoderma lineatum* (De Vill.) and *H. bovis* (L.). Applications were made by high pressure spray at 0.25% concentration, and by a localized "pour-on" technique (details of which are described) utilizing, in the definitive tests, a long-handled dipper to discharge 100 ml. of concentrated emulsion on the back of each test animal. Direct comparison was made of a thorough-coverage spray, at 0.25%, with a "pour-on" application consisting of 20 ml. of the 4 lb./gal. E.C. in 100 ml./head (equivalent to 1 gal. 0.25% spray-mix or 9.6 gm. actual Ruelene). The spray gave 80% and the "pour-on" gave 99% control (8 calves/treatment, 25.4 grubs/head in untreated controls). Equivalent but slight erythrocyte cholinesterase depression was detected in both treated groups. Slight weight gain differentials favored the treated groups but without significance. A group of 49 calves similarly treated by the "pour-on" technique demonstrated 96% to 98% reduction as compared with 45 well-infested, untreated controls. In another "pour-on" test 14 calves treated at a 10 ml. dosage showed 85% to 88% reduction; another 14 calves treated at a 20-ml. dosage showed 98% reduction; and 9 calves treated at a 40-ml. dosage showed 100% reduction in the number of grubs present as compared with 11 untreated controls. Some mild skin irritation was seen in some heavily dosed dairy cattle, but none occurred on any of the Hereford cattle treated in these tests.

Within the past 4 years a significant number of effective systemic agents have been developed for the control of cattle grubs, *Hypoderma lineatum* (De Vill.) and *H. bovis* (L.). These agents have been administered orally as single or multiple doses, as injections, or as sprays. While the current procedures represent a tremendous advance over the methods available a decade earlier, sufficient limitations remain to encourage the search for superior materials and superior modes of administration.

In 1959 The Dow Chemical Company announced the availability of a new organic phosphate compound, Ruelene® (4-*tert*-butyl-2-chlorophenyl methyl methylphosphoramidate), and presented data (Dow 1959, McGregor *et al.* 1959) showing high efficacy of this material, applied as sprays and otherwise, against cattle grubs.

The first practical systemic material applied as a spray was Bayer 21/199 (Co-Ral®) (Brundrett *et al.* 1957). Results obtained with this material have been generally good but have been somewhat erratic (Rogoff *et al.* 1960). The manufacturer emphasizes the necessity of complete coverage at high pressure under relatively ideal conditions of spray procedure (Skaptason 1959), though there are data that indicate that partial spraying can be as effective as total spraying (Rogoff *et al.* 1960), and that low pressures may be as effective as high pressures (Khan *et al.* 1959). Shortly after Ruelene became available, per-

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sonnel of the Dow Chemical Company investigated possibilities of using this material at high concentration and low volume, with application simply by pouring the insecticide on the backs of cattle. The authors felt that the technique was sufficiently promising to warrant extensive work on this method. This paper deals with Ruelene compared as spray applications over the entire body and as localized "pour-on" applications, details of which will be described. A companion paper will describe results of low-level, free-choice application in a mineral mixture administered in a manner comparable to that described by Rogoff & Kohler (1959).

MATERIALS AND METHODS.—The Ruelene used in these tests was a 4 lb./gal. emulsifiable concentrate formulated by the Dow Chemical Company. In the various tests described herein, this material was used either without further dilution, with slight dilution with water, or as normal sprays with relatively large quantities of water. Sprays were applied with a 200-gal. Evans orchard sprayer, at approximately 400 p.s.i. During spraying, animals were crowded into a small enclosure. The spray technique appeared to be excellent.

The "pour-on" methods utilized the emulsifiable concentrate either undiluted or slightly diluted (ranging from 1:2.5 to 1:24), using a graduated cylinder or a beaker. The fluid was poured directly onto the back of the animal being treated (fig. 1). In the definitive treatments used in the field, a 100-ml. beaker was attached to a stick by means of wire to form a long-handled dipper. It was then possible to use the dipper without getting any of the material on the operator. The operator simply moved down a line of animals held in a chute, dipping 100-ml. at a time from a plastic pail, and pouring the material over the back of each animal. The milk-colored emulsion remained visible on the back of the animal during the treatment procedure. This "pour-on" procedure, utilizing a dipper, is a remarkably easy method of treating animals, certainly the simplest method yet experienced. As an example of the speed and simplicity involved, it is worth referring to field notes of November 24, 1959. A long chute was filled with 40 calves. Equipment consisted of the 100-ml. dipper and the plastic pail containing the insecticide. Each animal was treated from the top of the shoulders to the hips (a distance of 24 to 26 inches), by moving the dipper back and forth 3 to 4 times as the emulsion was applied on each side of the backbone. There was a small amount of runoff (generally less than 5%) on some of the calves. Treatment started at 10:09 a.m. and finished at 10:27 a.m. Forty calves were treated in 18 minutes, less than half a minute per calf. These calves were run out of the chute and an additional 10 calves were run in; this treatment was started at 10:34 a.m. and was finished by 10:38 a.m., also less than half a minute per calf. The time involved is equivalent to a high pressure spray operation exclusive of the time for readying the sprayer for action and cleaning it afterwards.

Erythrocyte cholinesterase determinations were made by a method slightly modified from that of Michel (1949).

DATA.—Before proceeding with treatment of infested cattle it appeared desirable to test various concentrations and formulations of Ruelene for possible undesirable effects on skin as well as on cholinesterase activity. A group of dairy cattle of various ages and weights were available for this phase of the study. The data obtained are shown in table 1. Treatment was begun on October 8, 1959, with some applications of the undiluted emulsifiable concentrate and with dilutions in water to bring the total quantity to 250 ml. of mix. Animals treated were Holsteins, Guernseys, Brown Swiss, and one Jersey. At 4 days posttreatment there were no obvious lesions. On the 11th day there was slight roughening of the skin of the Jersey animal. On the 18th day the roughening on the Jersey had become more severe and there was some degree of scabbing. Irritation was still obvious on the 21st day. The scabs seemed to be clearing up by the 28th day, and by 42 days posttreatment there was no evidence of irritation on this animal. There was a slight roughening and hardening of the skin where treated on one of the Holsteins that had been dosed on October 19 with 100-ml.

FIG. 1.—"Pour-on" technique utilizing a 100-ml. beaker wired to a handle.

Table 1.—Erythrocyte cholinesterase activity in dairy cattle following topical application of Ruelene as 4 lb./gal. emulsifiable concentrate.

Treatment	No. of Cattle	Estimated Average Weight (lb.)	Cholinesterase Activity (%) (10 to 11 days posttreatment)
October 8, 1959			
10 ml. undiluted[a]	2	1,050	88
10 ml.: 240 ml. H_2O[a]	2	1,150	82
40 ml. undiluted[b]	2	1,100	62
40 ml.: 210 ml. H_2O[b]	2	1,200	76
Untreated	4	950	80
October 19, 1959			
100 ml. undiluted[c]	3	900	64
October 29, 1959			
40 ml. undiluted[b]	2	350	56
Untreated	2	750	97

[a] Equivalent to 0.125% spray at 1 gal./head (4.8 gm. actual Ruelene).
[b] Equivalent to 0.5% spray at 1 gal./head (19.2 gm. actual Ruelene).
[c] Equivalent to 1.25% spray at 1 gal./head (47.9 gm. actual Ruelene).

of undiluted emulsifiable concentrate. This slight hardening was seen 10 days after treatment, persisted on the 17th day, was somewhat evident yet on the 21st day, but had disappeared entirely by 31 days posttreatment. A slight roughening of the skin was seen on a cross-breed calf that had been treated with 40 ml. of the undiluted material on October 29. A slight roughening was seen 21 days later. On the basis of these preliminary tests, it was concluded that the skin irritation seen was such that, while objection would be raised in show animals about to be exhibited, no objection would be expected in the treatment of range animals. It was accordingly considered feasible to test this method further.

The data on cholinesterase activity shown in table 1 indicate that the higher concentrations apparently penetrate the skin sufficiently to produce depression. Thus it was considered that support had been obtained for the larger experiments on grub control which were to follow.

For a more precise evaluation of the efficacy of this material and these methods of administration, a group of steer calves was assembled from areas in South Dakota normally having heavy infestations of cattle grubs. These calves were lotted according to weight (average 380 lbs.) and source, and treated by spray (using standard procedures), by "pour-on" or held as controls. The "pour-on" technique utilized the dipper described earlier; 100-ml. of total mix were poured on each animal. Each 100 ml. contained 20 ml. of the emulsifiable concentrate, an amount equivalent to 1 gallon of 0.25% spray (9.6 gm. actual Ruelene). These calves were held in adjacent sheds and yards. Hay, grain, water, and a salt-bonemeal mixture were provided in a comparable manner for each lot. Brome hay was offered free-choice. A mixture of equal parts of oats and of corn was offered to each lot on the basis of 4 lb./calf/day. All of these calves were individually weighed at monthly intervals.

The data on infestation shown in table 2 indicate that at the concentrations used, the "pour-on" treatment provided 99% control as compared with the spray, which provided 80% control. The grub infestation was adequate (more than 25 grubs/animal) in this group of calves. It should be pointed out that, while the spray and "pour-on" treatments were designed to provide the same total quantity of Ruelene on each animal, actually the spray technique provided less per animal than the "pour-on." Essentially all of the material applied as "pour-on" stays on the animal, while with the spray there is a considerable degree of runoff. This should be borne in mind in interpreting these data as well as those that follow.

Studies of the erythrocyte cholinesterase activity, also shown in table 2, indicate that the degree of cholinesterase depression (at 23 and 64 days posttreatment) was almost identical for the two groups of animals. The degree of depression was moderate in both cases, but analysis of variance revealed no significant differences between groups. The studies on gain in weight appear to indicate an advantage for treatment in this instance, but analysis of variance did not indicate these differences to be significant. No skin irritation whatever was seen on any of these calves, regardless of treatment.

The "pour-on" technique was further tested on Hereford calves at substations of the Agricultural Experiment Station in western and central South Dakota. These data are shown in table 3. The Cottonwood-Antelope Station test involved treatment of the animals, using the dipper procedure, with 100 ml. of total mix containing 20 ml. of the emulsifiable concentrate. The average weight of these calves at the time of treatment was 372 lbs. These calves were herd-mates during the oviposition period by the *Hypoderma* spp. Excellent results were obtained, with between 96% and 98% reduction in cattle grubs.

The Reed Ranch test, results of which are also shown in table 3, involved treatment of calves at 10 ml., 20 ml., and 40 ml. of the emulsifiable concentrate diluted to 100 ml. with water. In this instance the 10-ml. and 20-ml. treatments were applied to heifers (with average weights of 408 and 390 lbs., respectively), the 40-ml. treatment was applied to steers (averaging 470 lbs.), while the bulls served as controls. Previous data have indicated no obvious differences in numbers of grubs in the different

Table 2.—Infestation, erythrocyte cholinesterase, and weight gain of steer calves following Ruelene treatment by total coverage spray or localized "pour-on" administered December 15, 1959.

Criteria	Treatment[a]		
	0.25% Spray	20 Ml. "Pour-on"[b]	Untreated
Infestation			
Calves infested	5	1	6
Grubs/calf (range)	0–23	0–1	0–57
Average grubs/calf	5.0	0.1	25.4
Reduction (%)	80	99	—
Erythrocyte cholinesterase			
January 7, 1960 (23rd day)			
Range[c]	0.31–0.54	0.26–0.48	0.30–0.59
Average[c]	0.43	0.36	0.45
Activity (% initial)	83	82	92
February 17, 1960 (64th day)			
Range[c]	0.44–0.69	0.35–0.61	0.36–0.66
Average[c]	0.55	0.47	0.54
Activity (% initial)	106	107	110
Average weight gain/calf			
Initial weight (lb.)	374	391	374
Daily gain (lb.)[d]	1.82	1.78	1.57
Per cent of initial weight	61	57	53

[a] Eight calves used in each category; sum of three extractions, Feb. 5, Mar. 16, and Apr. 13.
[b] Poured 100 ml. of aqueous mixture containing 20 ml. Ruelene, E.C., 4 lb./gal. Equivalent to 1 gal. 0.25% spray mix (9.6 gm. actual Ruelene).
[c] ΔpH units/hour. Not significant at 5% level.
[d] Not significant at 5% level. Test ran for 125 days.

Table 3.—Effectiveness of Ruelene emulsions at various dosages as 100 ml. localized "pour-on" applications administered to calves, November 24, 1959.

Criteria	Treatment and Counts by Palpation							
	10-Ml. Dosage[a]		20-Ml. Dosage[b]		40-Ml. Dosage[c]		Untreated	
	Feb.	Mar.	Feb.	Mar.	Feb.	Mar.	Feb.	Mar.
Cottonwood-Antelope Station Test								
No. treated			49	49			45	45
Infested (%)			6	8			100	100
Grubs/head (range)			0–23	0–39			6–72	3–71
Average grubs/head			0.6	0.9			36.2	26.3
Reduction (%)			98	96				
Reed Ranch Test								
No. treated	14	14	14	14	9	9	11	11
Infested (%)	21	43	7	14	0	0	64	100
Grubs/head (range)	0–4	0–8	0–2	0–2	0	0	0–18	2–28
Average grubs/head	0.6	1.4	0.1	0.2	0	0	4.9	9.4
Reduction (%)	88	85	98	98	100	100		

[a] 100 ml. aqueous emulsion containing 10 ml. Ruelene, E.C., 4 lb./gal. Equivalent to 1 gal. 0.125% spray mix (4.8 gm. actual Ruelene).
[b] Equivalent to 1 gal. 0.25% spray mix (9.6 gm. actual Rulene).
[c] Equivalent to 1 gal. 0.5% spray mix (19.2 gm. actual Ruelene).

sexes and therefore the sex of the calves did not influence the reliability of these tests. It is plain that as dosage was increased in this test, degree of control obtained also increased. The 10-ml. dosage provided between 85% and 88% control, the 20-ml. dosage provided 98% control, and the 40-ml. dosage provided 100% control. No evidence of irritation on the skin was seen on any of these animals. With the exception of the skin irritation of some of the severely challenged dairy animals used in the initial tests, no signs of toxicity were seen in any of the animals under study in this experiment. Identification of grubs extracted indicated an unusual predominance of *H. lineatum* over *H. bovis*. The ratio was roughly 85% *lineatum* to 15% *bovis*.

DISCUSSION.—It would appear from these data that Ruelene is an effective material for the control of cattle grubs when applied either as a spray or as a localized "pour-on" with the "pour-on" apparently superior to the spray. While no evidence of toxicity was seen, there is not sufficient data presented here to warrant a prediction that further work would be equally free of hazard.

Attention should be called to the fact that the dosages used in this research were not scaled to the individual weights of the experimental animals. This is necessarily, for reasons of practicality, the appropriate procedure for spray applications, though larger animals automatically receive larger dosages (but not in direct proportion) as a result of greater body surface to which spray can adhere. Further research is desirable to determine whether the dosage provided by the "pour-on" method should be scaled to the weights of animals undergoing treatment.

The "pour-on" method has proven so easy to employ and, in these instances so effective, that further work with Ruelene and with other materials by this method would be desirable. No other procedure has been utilized by the writers requiring so little effort and so little handling of the animals. It is simple and rapid, and it is accurate in that a known quantity of material is applied to each animal that is being treated. It also appears to require less total material than the spray technique. The small amount of runoff experienced could be further reduced by improvement of technique or by increasing the viscosity of the formulation. The "pour-on" method has the further advantage of being susceptible to use in connection with other routine husbandry operations, thereby not requiring a separate roundup. The marketing of a metal or plastic dipper with the insecticide might be feasible.

Confirmation of the data presented in this paper in large scale tests is regarded as highly desirable.

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Field Experiments on the Insecticidal Control of Insects Attacking Peas, Snap and Lima Beans[1]

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ABSTRACT

Methyl Trithion® (*O,O*-dimethyl *S*-(*p*-chlorophenylthio) methyl phosphorodithioate), dimethoate, phosphamidon, and Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide), gave high reductions of pea aphid (*Macrosiphum pisi* (Harris)) on peas. Dibrom® (1,2,-dibromo-2,2-dichloroethyl dimethyl phosphate), phosphamidon, Dilan® (1 part of 1,1-bis(*p*-chlorophenyl)-2-nitropropane plus 2 parts of 1,1-bis(*p*-chlorophenyl)-2-nitrobutane) and ethion showed considerable effectiveness against the potato leafhopper (*Empoasca fabae* (Harr.)) on snap beans; dimethoate gave best control. Sevin® (1-napthyl methylcarbamate) and DDT gave 95% to 100% reduction of corn earworm (*Heliothis zea* (Boddie)) injury to green beans. Lesser reductions were obtained with Methyl Trithion, ethion, dimethoate, Dylox® (dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate), Thiodan, and phosphamidon. On lima beans 98.9% to 99.4% clean pods were obtained by sprays of DDT and Sevin repeated at 10-day intervals. There was no significant difference between 1- and 2-pound per acre rates of application of Sevin and DDT against the corn earworm on lima beans.

There are at present adequately effective and approved insecticides for control of pests attacking peas and beans in Maryland. In observing commercial operations, pea growers are carrying out efficient control programs for the pea aphid, *Macrosiphum pisi* (Harris), on spring-planted peas. Malathion sprays, applied either by airplane or ground equipment, are generally used. Malathion is preferred because it is comparatively safe to apply, provides no residue hazard on the pea vine silage, is highly effective and is comparatively inexpensive. The only objection to malathion has been its tendency to cause a chlorosis of peas when applied during hot weather or in heavy dosages. The extent of retardation in such instances has not been determined. Peas usually appear to recover in 7 to 10 days.

The pea aphid and the seed-corn maggot, *Hylemya cilicrura* (Rond.), are the only serious pests that attack spring peas, and the smooth seeded or Alaska type appears to be immune to maggot attack. Seed treatments with an insecticide, preferably dieldrin, are generally used and provide considerable protection of wrinkled peas against seed-corn maggot. Prolonged periods of cold wet weather after planting reduce the effectiveness of seed treatment. Wrinkled peas are usually planted later than smooth seeded peas to avoid this condition. Fall peas are attacked by pea aphid and several species of Lepidoptera, most important of which is the corn earworm, *Heliothis zea* (Boddie). For these, additional treatments (usually DDT) are necessary. Fall peas have not been planted extensively in recent years in the area.

It appears that in commercial production of snap and lima beans growers are not using insecticides as efficiently as on peas. They are successful in controlling the Mexican bean beetle, *Epilachna varivestis* Muls., but heavy losses have been observed from infestations of spider mites, mostly the two-spotted spider mite (*Tetranychus telarius* (L.) the green cloverworm, *Plathypena scabra* (F.), and the corn earworm. Growers tend to wait until they see obvious injury from these pests before treatment is applied, by which time serious damage is done. This results not so much from an indifferent attitude on the part of growers as from their inability to determine the presence of the insects early enough. Preventive insecticide programs are necessary particularly on fall grown beans. The work reported here was carried out to obtain information on a number of insecticides and on the extent of injury and value of treatments to be used to impress growers with the importance of preventive insecticide programs on late season beans.

Four experiments were carried out. The experiment on peas was undertaken primarily to obtain information on the residues and effectiveness of phosphamidon and dimethoate; a mid-season snap bean experiment was for the purpose of obtaining information on several insecticides for control of potato leafhopper, *Empoasca fabae* (Harr.). Late season plantings were used for determining effectiveness of insecticides against the corn earworm on snap beans and on lima beans.

EXPERIMENTS AND RESULTS.—*Peas.*—Perfect Freezer 66 variety of wrinkled peas was planted April 15 in plots 5 by 80 feet. Seven insecticidal treatments, each replicated three times in randomized blocks, were applied on June 4. All insecticides were in the emulsifiable form and applied in 25 gallons of water per acre with a broadcast sprayer (Ditman & Burkhardt 1952). Aphid populations were estimated by the sweep method just before spraying and again 4 days later. The primary purpose of the experiment was to obtain residue information for some of the insecticides used over a period that extended beyond the time when peas were normally at processing maturity. Only a part of each plot was harvested to obtain samples of fruit that were canned and had the flavor evaluated. Nature of treatment, population estimates, and flavor evaluations are given in table 1. Methyl Trithion® (*O,O*-dimethyl *S*-(*p*-chlorophenylthio) methyl phosphorodithioate), dimethoate, phosphamidon and Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide) at the dosages used all apparently gave excellent pea aphid control and caused no off-flavors of the canned peas. Reduction of aphids on check plots was attributed to insecticidal drift since heavy populations developed on other peas about one-fourth mile away. The effect of drift of the highly effective newer insecticides is a serious problem in repli-

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Table 1.—Insecticidal treatments applied to peas, population estimates, and flavor evaluations.

Insecticide	Amount per Acre: Pints Emulsion Concentrate	Amount per Acre: Actual Toxicant (lb.)	Pea Aphids per Sweep: June 4	Pea Aphids per Sweep: June 8	Flavor Value[a]
Methyl Trithion	4.0	1.00	17	0.06	3.5
Dimethoate	0.5	0.25	24	.00	3.7
	1.0	0.50	32	.00	4.0
	2.0	1.00	20	.00	3.2
Phosphamidon	0.5	0.25	16	.00	3.8
	1.0	0.50	25	.06	3.8
Thiodan	3.0	0.75	26	.12	3.5
Check	—	—	36	.00	2.9[b]

[a] In this and following tables:
5 = better than standard
4 = standard
3 = below standard, no noticeable off-flavor
2 = definite off-flavor
1 = not acceptable

[b] In these results check was significantly poorer than all treatments.

cated small plot experiments that should be considered in the plan or design of an experiment and in interpretation of results. The effect of drift has been particularly obvious the past two seasons in pea aphid experiments where the systemic insecticides, phosphamidon and dimethoate, have been used (Young & Ditman 1959b). Wide separation of plots, use of large plots, and the use of barrier crops are possible solutions to the problem; however, the measures that can be taken by the investigator may be limited by the facilities available so that this factor must be considered in evaluating results.

Early Snap Beans.—The Tenderlong 15 variety of snap beans was planted June 9 so as to grow at the time a high infestation of potato leafhopper could be expected and after the spring migration of adult Mexican bean beetles from hibernation. Treatments were replicated four times on plots containing four rows 40 feet long and with single untreated rows between plots. Seven insecticidal treatments were applied on June 30 and July 17. All insecticides were used in the emulsifiable form and applied in 25 gallons of water per acre with an improved spray boom (Burkhardt & Ditman 1956). Leafhopper populations were estimated by counting the number of nymphs on 100 leaves selected at random on the inner two rows of each plot. Since an insignificant Mexican bean beetle infestation occurred, populations were not estimated, but pods at harvest were examined for scars caused by the feeding of this insect. Beans were harvested on July 30 and August 5. Yields were recorded and fruit of the first harvest canned for flavor evaluation. Nature of treatments and the results are given in table 2. At the dilutions used all insecticides gave considerable reduction of potato leafhopper nymphs on the bean plants. Dimethoate at 0.5 or 1.0 pound per acre gave best control. There were no off-flavors of canned fruit caused by any of the treatments.

Late Snap Beans.—Because of the abnormally early spring emergence of corn earworm moths and the extremely dry early summer favorable to rapid buildup of earworm populations a heavy late-season infestation of earworm was expected. Consequently, snap beans and lima beans were planted for the evaluation of insecticidal treatments against this insect. A second crop of Tenderlong 15 variety of snap beans was planted August 12. The entire field of snap beans was sprayed with malathion for control of Mexican bean beetle on September 4. A single application of all treatments for control of the corn earworm was made September 22 at the time the first fruit was in the pin stage. Treatments were to 4-row, 0.01-acre plots randomized in blocks replicated four times. All insecticides except Sevin® (1-napthyl methylcarbamate) and Dylox® (dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate) were used in the emulsifiable form, and were applied in 25 gallons of water per acre with an improved or "Slosser" type boom with three nozzles per row.

Table 3.—Treatments applied for control of corn earworm on snap beans and estimates of resulting pod infestation.

Insecticide	Amount per Acre: Concentrate (lb. or pt.)	Amount per Acre: Actual Toxicant (lb.)	Total No. Pods Examined	Per Cent: Infested	Per Cent: Control	Flavor Values
Sevin w.p.	2 (w.p.)	1.0	402	1.4	96.4	4.0
	4 (w.p.)	2.0	410	0.0	100.0	4.0
DDT	4	1.0	416	1.6	95.9	4.0
	8	2.0	407	0.7	98.2	4.0
Methyl Trithion	2	1.0	381	32.0	18.5	3.9
Ethion	1	0.5	390	28.9	26.4	4.0
Dimethoate	2	1.0	415	14.2	63.8	4.1
Dylox w.p.	1.25 (w.p.)	1.0	408	10.0	74.5	4.0
Thiodan	4	1.0	428	5.3	86.5	4.0
Sevin (Flowable)	2	1.0	413	0.0	100.0	4.1
Phosphamidon	1	0.5	377	22.5	42.7	4.0
	2	1.0	389	12.3	68.7	4.0
Check	—	—	409	39.3	—	4.0

Table 2.—Treatments applied to early snap beans against potato leafhopper, average yields per plot, and per cent beans scarred by Mexican bean beetle.

Insecticide	Amount per Acre: Pints Emulsifiable Concentrate	Amount per Acre: Actual Toxicant (lb.)	Leafhopper Nymphs per 100 Leaves: July 15	Leafhopper Nymphs per 100 Leaves: July 29	Pounds per Plot	Bean-Beetle Scarred Pods (%): July 30	Flavor Values
Dimethoate	0.5	0.25	1.7	0.2	25.6	0.7	3.8
	1.0	0.50	.2	0.0	24.8	0.5	3.8
	2.0	1.00	.2	0.0	26.9	1.2	3.7
Dibrom®[a]	1.0	1.00	4.5	2.7	24.9	1.0	3.6
Phosphamidon	1.0	0.50	6.5	6.5	25.5	1.7	4.0
Dilan®[b]	2.0	0.50	5.2	1.5	23.6	0.2	3.6
Ethion	1.0	—	9.2	3.7	26.4	1.5	3.7
Check	—	—	52.8	27.2	23.8	3.2	3.8

[a] 1,2,-dibromo-2,2-dichloroethyl dimethyl phosphate.
[b] One part of 1,1-bis(*p*-chlorophenyl)-2-nitropropane plus 2 parts of 1,1-bis(*p*-chlorophenyl)-2-nitrobutane).

Table 4.—Results of insecticides applied to Fordhook 242 lima beans and estimates of their effectiveness against the corn earworm.

Insecticide	Amount per Acre: Concentrate (pt. or lb.)	Amount per Acre: Actual Toxicant (lb.)	Total No. Pods Recovered[a]	Clean Pods (%)	Average Number of Clean Pods per: Plant	Average Number of Clean Pods per: Foot of Row	Shelled Beans per Plot (lb.)	Flavor Values
DDT	4	1.0	1,121	98.9	13.8	23.3	14.2	3.8
	8	2.0	948	99.8	11.8	15.9	12.1	3.8
Sevin (w.p.)	2	1.0	1,310	99.0	16.2	20.8	15.4	3.9
	4	2.0	1,226	99.1	15.1	23.2	15.7	3.9
Phosphamidon	1	0.5	728	60.0	5.4	6.6	5.9	3.7
	2	1.0	935	79.0	9.2	14.7	11.0	3.9
Methyl Trithion	2	1.0	853	58.6	6.2	7.3	4.4	3.4
Ethion	1	0.5	596	36.5	2.7	3.3	3.0	3.7
Sevin (flowable)	2	1.0	1,116	99.4	13.8	25.5	15.5	4.0
Dimethoate	2	1.0	712	73.7	6.5	9.9	7.0	3.8
Thiodan	4	1.0	206	94.6	2.4	4.0	3.4	3.8
Dylox (w.p.)	1.25	1.0	1,227	47.6	13.0	14.9	8.7	3.9
Check	—	—	216	85.2	1.2	1.8	1.4	3.8

[a] Includes injured pods found on the ground under plants.

Observations on earworm infestation in the pods were made on 10 plants selected at random from the inside rows. Nature of treatments and the results are given in table 3.

At the concentrations used all insecticides showed some effectiveness against corn earworm larvae on snap beans. The performance of Sevin and DDT was outstanding. Only Sevin wettable powder at the 2-pound rate and Sevin flowable at the 1-pound rate gave complete control in the samples observed. None of the treatments caused off-flavor of the canned fruit.

Late Lima Beans.—The Fordhook 242 variety of lima beans was planted July 17. The entire field was sprayed with malathion at the rate of 1 quart emulsifiable concentrate per acre on August 14 and 21 for control of spider mites and Mexican bean beetle. Five applications of the same treatments as applied to late snap beans were applied in the same way to the lima beans on August 26, September 4, 15, 23, and October 2. All treatments were replicated four times on 2-row plots in randomized blocks. Plots were separated from each other by single untreated rows. Estimates of corn earworm infestation and damage were made on duplicate 10-plant samples from each plot on October 13 and 14. Because developing lima bean pods when fed upon by corn earworm larvae often fall from the plant in the field, counts were made of decomposing pods on the ground as well as the number of infested and uninfested pods on the plants. All plots were harvested on October 21. Yields were recorded and samples canned for flavor evaluation. The results of observations on extent of damage, yields of shelled beans, and flavor evaluations of canned beans are given in table 4.

Of the insecticides observed only Sevin and DDT show desirable effectiveness against the corn earworm on lima beans. On Fordhook 242 limas, Thiodan caused chlorosis, dwarfing of plants, and considerable reduction in yield. This was the first such injury that had been observed on lima beans. In previous tests at a lower dosage no apparent phytotoxicity had been observed (Young & Ditman 1959a).

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A 5-Year Report of Observations in the Japanese Beetle Control Area at Sheldon, Illinois[1]

W. H. Luckmann and G. C. Decker[2]

Abstract

Beginning in the spring of 1954 and continuing through 1958, dieldrin was applied by the Division of Plant Pest Control Agricultural Research Service, U. S. Department of Agriculture, and the Illinois Department of Agriculture for suppression of a local infestation of Japanese beetles (*Popillia japonica* New.) near Sheldon, Illinois. During this 5-year period dieldrin was broadcast over 17,844 acres of Illinois farm land at a dosage of 2 or 3 pounds of toxicant per acre. Records were obtained on the effects of the treatment on the Japanese beetle, other insects, earthworms, and livestock.

One treatment of dieldrin gave excellent control of Japanese beetle larvae during the 5 years covered by this study. Many other insects that come in occasional or frequent contact with the treated soil were controlled for periods ranging from 1 to 5 years. Populations of a few economic insects increased following the treatments of dieldrin, but the increase was not great enough to warrant additional control measures.

Some predators were adversely affected or eliminated by the dieldrin, whereas other predators and parasites appeared not to be harmed. The treatment did not eliminate earthworms.

Farm livestock confined to pastures or farm lots treated by airplane with 20 or 30 pounds of 10% granulated dieldrin per acre showed no ill effects. However, poisoning and death occurred in livestock, particularly sheep, exposed to drift from aerial sprays of 3 pounds of dieldrin per acre.

The magnitude of dieldrin residues found on forage treated with granulated dieldrin depended to a considerable extent on the condition of the forage at the time it was treated.

In 1916, a few conspicuous green beetles, found near Riverton, New Jersey, were identified as the first Japanese beetles, *Popillia japonica* New., ever found in the United States. From this small infestation the insect multiplied and spread throughout large areas in the eastern part of this country. Quarantine restrictions, while helpful, did not successfully confine the beetle to eastern states, and numerous isolated infestations appeared in the southeastern states and the Midwest, including Illinois. In 1953, a large infestation of Japanese beetles was found along the Illinois-Indiana border near Sheldon, Illinois. A survey that year showed that 10,000 acres of some of the best farm land in Illinois were infested.

The infested farms produce heavy crops of corn and soybeans and lesser amounts of alfalfa, red clover, and small grain. The area also supports limited milk, egg, poultry, beef, pork, and sheep production. Since surveys showed that the beetles preferred corn and soybean fields and that egg laying was practically negligible in small grain, forage, and permanent sod, the urgency for some kind of action in a high value agricultural area fairly well defined the type of program necessary. Thus, large-scale broadcast application of a soil insecticide to entire farmsteads seemed desirable.

A control program to retard the spread of this pest was begun by the Plant Pest Control Division, Agricultural Research Service, U. S. Department of Agriculture, and the Illinois Department of Agriculture early in 1954 when dieldrin was applied broadcast to a block of 1,535 heavily infested acres. Additional areas became infested each year until in 1958, beetles were found on approximately 50,000 acres of Illinois farm land around Sheldon. Likewise, treatments were continued each year and by 1958, 17,844 acres, including the villages of Sheldon and Effner, had been treated with dieldrin. Prior to initiation of the control program in 1954, the cooperating federal and state agencies requested that the research staff of the Illinois Natural History Survey study the area continuously to determine the effects of the treatment on insects, livestock, and other animals.

Procedures.—Treating for control of Japanese beetles at Sheldon was begun in April, 1954, with airplane applications of sprays and granules at a dosage of 3 pounds of technical dieldrin per acre. Treatment was made with 3 gallons of Shell solvent TS-28R containing 3 pounds of technical dieldrin per acre or 30 pounds of 10% dieldrin clay granules per acre. In 1955, only granules were used at a dosage of 2 or 3 pounds of dieldrin per acre. Granules were applied during 1956, 1957, and 1958, and the dosage remained at 2 pounds per acre. In addition, roadsides through the infested area were sprayed with 1 pound of DDT per acre, and an area of 300 acres divided equally into three sections was treated with aldrin, heptachlor, and chlordane granules in 1956. Once an area was treated, it was not retreated. In plowed fields the insecticide was often worked into the soil shortly after application; in meadows and permanent pastures the soil was not disturbed until the following or some subsequent year if at all.

From 1954 through 1957, all treatments were applied to the most heavily infested fields so that, following the program in 1957, a block of 9,649 acres had been treated in Illinois. (Approximately 3,460 acres were treated in the adjacent state of Indiana, resulting in a solid block of 13,109 acres in the two States.) The treatment made in 1958 consisted of a one-half mile barrier strip on three sides of the total area known to be infested. The fourth or east side along the Illinois-Indiana border, was not treated except as portions had been treated in previous years. This treated U-shaped band, 27½ miles long and one-half mile wide, is roughly 3 to 5 miles from the outer edge of the solid block treated during the 1954–1957 period. When dieldrin was applied to this strip, as with other areas around Sheldon, everything was treated including farms, houses, streams, and roads. Information regarding treatments during the 5-year period is given in table 1.

Estimating the numbers of insects in treated and untreated areas was done by sweeping with a 15-inch net,

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Table 1.—Time of application, number of acres treated, and dosage of dieldrin applied in the Japanese beetle control area at Sheldon, Illinois from 1954 through 1958.

Dates of Application	Number of Acres Treated	Actual Dieldrin per Acre (lb.)	Type of Formulation Applied by Airplane
April 12–May 11, 1954	1,535	3	Sprays[a] and granules[b]
April 15–May 6, 1955	2,860	2 and 3	Granules[b]
April 15–April 24, 1956	2,414	2	Granules[b]
April 10–April 17, 1957	2,840	2	Granules[b]
March 29–April 9, 1958	8,195	2	Granules[b]
Total acres treated	17,844		

[a] Three pounds technical dieldrin in 3 gals. Shell solvent TS-28R.
[b] Ten per cent dieldrin clay granules.
Note: Approximately 3,460 acres treated in Indiana adjacent to the infestation in Illinois.

soil sampling, individual plant examination, plant dissection, Berlese processing of grass clumps and debris, planting of trap crops, and visually estimating numbers of insects by walking through fields and along fence rows. Generally, no attempt was made to determine quantitatively the exact density of an insect population or minor differences in the number of insects present in any area. The insecticide was recorded as having no effects when a specific insect was found to be about equally abundant in both the treated and untreated areas during the season or from year to year. If, on the other hand, an insect was obviously more numerous or practically absent in one area and not in another, the difference was attributed to effects of the insecticide. Thus, records were made of the obvious differences in numbers of insects and the duration of these differences.

It was apparent even before the treatment began that some attention must be given to livestock in the area since the effects of such a large-scale application of dieldrin to farm land and farm homesteads were not known. Some farmers living in the treated area were asked to supply samples of milk from cows placed immediately onto treated pastures or to release some animals onto treated pastures at varying intervals following application. Practically all of the farms with livestock or poultry were inspected and many of these inspections were made with the assistance of a veterinarian. Whenever animals became ill or died, detailed records were obtained regarding the conditions causing illness or death. Small animals, as well as tissue and organs of some large animals, were examined in the laboratory. Some milk and tissue samples were analyzed for dieldrin.

As a supplement to the observations on livestock in 1954 and 1955, residue data were obtained from several fields representing various types of forage and various ecological conditions at the time of application of the insecticide. Likewise, some forage samples were taken from pastures containing sick or dead livestock. Samples of forage were obtained by hand clipping 1,000 grams of plant material and quick-freezing the clippings. In some instances samples were not taken at random since it was important to obtain clippings only from fence rows where contamination by drift was suspected. Forage samples were analyzed for dieldrin.

RESULTS AND DISCUSSION.—*Insects.*—Naturally many insects were affected in one way or another as a result of the treatment of such a large area with a high dosage of insecticide. Many common insects that come in occasional or frequent contact with the soil were quickly eliminated from the treated area, and some species were still not present 5 years after treatment. Some of the more common insect problems of field and forage crops did not occur on the farms that were treated. On the other hand, populations of some insects increased noticeably even though many very important predators appeared not to be greatly affected by the insecticide. A list of some insects studied and the effects of the dieldrin on these insects is presented in table 2.

One of the most interesting effects caused by the insecticide concerned the Japanese beetle. The treatment applied early each spring killed only about 50% of the larvae in the soil at the time of treatment. Occasionally large numbers of poisoned grubs would crawl to the surface of the soil and die, but many grubs survived and successfully pupated. Adult beetles were usually present in a treated area during late June, July, and early August following the treatment, and it appeared that the insecticide had no effect on the adults laying eggs. However, larvae hatching from eggs laid during the summer in the treated soil were killed. The soil treated in the spring of 1954 with 3 pounds of dieldrin per acre and repeatedly plowed and cultivated was still free of Japanese beetle larvae in the fall of 1958. These observations support the research of Polivka (1956) who reported that dieldrin at 1 or 5 pounds per acre was 100% effective for at least 3 years.

Larvae belonging to *Phyllophaga* spp. and *Cyclocephala* spp. were fairly abundant in the fields of corn and soybeans around Sheldon. Spots in some fields occasionally contained as many as 10 to 15 per cubic foot of soil. Many of the observations made on the presence and abundance of white grubs and Japanese beetle larvae were obtained while walking behind the plow during spring and fall plowing operations. Records taken in the spring and fall of 1958 showed that the areas treated in 1954 with 3 pounds of dieldrin per acre were still free of larvae of *Phyllophaga* spp. and *Cyclocephala* spp.

Two species of rootworms, the southern corn rootworm, *Diabrotica undecimpunctata howardi* Barber, and the northern corn rootworm, *Diabrotica longicornis* (Say), are regular inhabitants of cornfields in east-central Illinois. The dieldrin treatments were quite effective in reducing the numbers of these insects. Larvae of the southern corn rootworm were found in treated fields the second year following treatment, but larvae of the northern corn rootworm were not found until the fourth growing season or the third year after the treatment.

Closely related species of flea beetles were affected quite differently by the dieldrin. The toothed flea beetle, *Chaetocnema denticulata* (Ill.), was practically nonexistent in corn for two seasons, whereas the sweetpotato flea beetle, *Chaetocnema confinis* Crotch, was regularly collected in treated areas though in much lower numbers than outside the treated areas. Some *Epitrix* spp. seemed to be unaffected by the treatment and they were abundant everywhere.

Evidence of effects of the dieldrin on the corn flea beetle, *Chaetocnema pulicaria* Melsh., was obtained by collecting with a sweep net and by planting sweet corn susceptible to bacterial wilt as an indicator crop. During 1954 and again in 1955, Victory Golden sweet corn was interplanted with field corn in three fields in each study

Table 2.—**Relative abundance of some insects in large areas treated with dieldrin, based on numbers of insects found in adjacent untreated areas, in the Japanese beetle control area at Sheldon, Illinois.**

Taxa	Insect Populations in Treated Areas After Treatment[a]				
	Same Year	First Year	Second Year	Third Year	Fourth Year
ENTOTROPHI					
Campodeidae	0	0	–	S	S
Japygidae	–	–	S	S	S
COLLEMBOLA					
Entomobryidae	0	0	–	S	S
Sminthuridae	0	–	S	S	S
ORTHOPTERA					
Melanoplus spp.	0	0	S	S	S
Gryllidae	0	0	–	S	S
Tettigoniidae	–	S	S	S	S
HEMIPTERA					
Lygus lineolaris	–	+	S	S	S
Adelphocoris lineolatus	–	+	S	S	S
Adelphocoris rapidus	–	+	S	S	S
Chlamydatus associatus (Uhl.)	–	–	–	–	S
Orius insidiosus	S	S	S	S	S
Nabis ferus (L.)	S	S	S	S	S
Blissus leucopterus	0	0	–	–	S
Macrosiphum pisi	S	+	S	S	S
Philaenus leucophthalmus	0	–	–	S	S
Draeculacephala spp.	S	S	S	S	S
Macrosteles spp.	+	+	S	S	S
Empoasca spp.	+	+	+	S	S
Agalliinae spp.	+	+	+	S	S
Paraphlepsius spp.	+	+	S	S	S
Psammotettix spp.	S	+	S	S	S
Nesosteles spp.	–	–	–	S	S
Graminella nigrifrons (Forbes)	+	+	+	S	S
Polyamia inimica (Say)	+	S	S	S	S
Delphacinae spp.	+	+	S	S	S
LEPIDOPTERA					
Heliothis zea (Boddie)	S	S	S	S	S
Peridroma margaritosa (Haw.)	0	–	S	S	S
Plathypena scabra (F.)	S	S	S	S	S
Pyrausta nubilalis	+	+	+	+	+
COLEOPTERA					
Coccinellidae	S	S	S	S	S
Carabidae	0	–	–	S	S
Hypera nigrirostris	0	0	–	S	S
Hypera punctata	0	–	–	S	S
Epitrix spp.	S	S	S	S	S
Chaetocnema confinis	–	–	S	S	S
Chaetocnema denticulata	0	0	S	S	S
Chaetocnema pulicaria	0	0	S	S	S
Diabrotica longicornis	0	0	0	–	S
D. undecimpunctata howardi	0	0	–	S	S
Systema blanda Melsh.	0	0	–	S	S
Popillia japonica	0	0	0	0	0
Phyllophaga spp.	0	0	0	0	0
Cyclocephala spp.	0	0	0	0	0
Glischrochilus spp.	+	+	+	+	+

[a] 0 = insects practically absent; – = fewer than in untreated areas; S = same as in untreated areas; + = greater than in untreated areas.

area. Farmer cooperators who were planting corn on the same day were asked to partially fill a planter box with sweet corn seed and plant the seed right along with their field corn seed. In 1954, 29% of the sweet corn plants growing in the untreated study area showed symptoms of wilt. Eleven per cent of the plants showed wilt symptoms in 1955. No incidence of wilt occurred in the areas treated with 3 pounds of dieldrin during these two years. Corn flea beetles and bacterial wilt were observed during 1956 in one area treated in 1954.

Grasshoppers, *Melanoplus* spp., were very numerous in the region in 1954, 1955, and 1956, but were practically absent in treated areas. In 1954, it was estimated that some fields of alfalfa and red clover in the untreated sections contained as many as 30 grasshoppers per square yard while treated sections averaged less than one-sixth hopper per square yard. In some instances, untreated and treated fields were no farther apart than the width of a country road. The treatment gave very effective control of some grasshoppers and some other common Orthoptera for a period of 2 years, *i.e.*, the year of treatment and the year following treatment. High-flying adults moved into the treated areas every year during late summer but sizeable numbers of nymphs were not observed until the second year following treatment.

Long-horned grasshoppers, family Tettigoniidae, were not eliminated as were *Melanoplus* spp., but the numbers observed and collected in the treated area were less than in adjoining untreated areas.

The chinch bug, *Blissus leucopterus* (Say), was particularly destructive during 1954 and 1955 in the vicinity of Sheldon. A few adult bugs were collected in late fall of 1954 and 1955 in the treated areas, while chinch bugs were abundant and very destructive in untreated areas adjacent to the treated farms. Each fall some adult bugs moved into the treated areas to hibernate in prairie grasses, but successful overwintering and reproduction the following year did not occur until the third season. Even then, the number of live insects was less than in untreated areas. As an example, counts made in the spring of 1958 showed no live bugs in the area treated in 1957, 3.6 live bugs per square foot in the 1956 area, 16.2 per square foot in the 1955 area, and 20 per square foot in the 1954 area. The adjoining untreated areas averaged 30.5 bugs per square foot.

Nymphs of the meadow spittlebug, *Philaenus leucophthalmus* (L.), were completely eliminated the year of treatment, and the effects of the treatment were still obvious the third season when a reduction of 56% was observed in the treated fields. However, adult meadow spittlebugs were usually quite numerous in treated fields around the first of September each year.

Dieldrin affected some leaf weevils about the same as it affected nymphs of the spittlebug. Larvae of the clover leaf weevil, *Hypera punctata* (F.), and the lesser clover leaf weevil, *Hypera nigrirostris* (F.), were effectively controlled the year of treatment, the year following treatment, and partially controlled the second year following treatment.

The spectacular reduction of leaf weevil larvae obtained with granulated dieldrin is very interesting. The daytime habits of the larvae seeking to hide in the debris and loose soil on the ground help to explain how the larvae were killed in the years following a treatment with dieldrin. It is also quite possible that treated soil is splashed by rain or blown by wind onto debris or onto the foliage or lower leaves of some plants.

There was quite a reduction in the number of plant bug nymphs the year of treatment but only a short or temporary reduction in the number of adult bugs. The year fol-

lowing a treatment, the second year, there tended to be slightly higher numbers of adult bugs, mostly tarnished plant bugs, *Lygus lineolaris* (P.deB.), in the treated areas. Numbers of *Adelphocoris lineolatus* (Goeze) and *Adelphocoris rapidus* (Say) also increased slightly the second year. The treatment appeared to have no effect the third year.

The pea aphid, *Macrosiphum pisi* (Harr.), did not appear to be greatly affected by the dieldrin the year of application and about equal numbers were found in treated and untreated areas. However, as with the plant bugs, there tended to be slightly higher numbers of aphids in the treated areas the second year. No effects were noted after the second year. These observations on the pea aphid do not completely agree with similar observations made by Wilson & Davis (1952). Wilson showed an immediate increase of aphids following foliage application of one-half pound of dieldrin. It is true that some treated fields had heavy infestations of aphids, and during 1954, records indicated that the insecticide may have caused an increase in the numbers of pea aphids in some fields of alfalfa. However, subsequent observations during the succeeding 4 years showed there was no consistent increase in pea aphids following application of dieldrin. Wilson & Davis (1952) applied dieldrin as a foliage spray to alfalfa, whereas the Sheldon study was based on the effects of a soil treatment. Perhaps the difference in type of application accounts for the variance in results.

The yearly observations recorded in the Japanese beetle area at Sheldon consistenly showed that while the dosage of dieldrin was not always sufficient to kill the adults of many insects, newly hatched nymphs or larvae were killed by infinitesimal amounts of the insecticide. This was demonstrated by the posttreatment effects on the immatures of many insects.

The treatment had an advantageous effect on most species of leafhoppers and the alfalfa fields at Sheldon dramatically confirmed the report by Wilson & Davis (1952)that increases in potato leafhoppers occur following use of dieldrin. Considering all leafhoppers, there was a definite increase in total numbers the year of treatment, and some effects even the third year. *Empoasca* spp., Agalliinae spp., and *Graminella* spp. were still slightly more abundant in the treated area the third year following application. *Empoasca* spp. were more abundant on fruit trees in the treated area.

The treatment increased the number of European corn borers (*Pyrausta nubilalis* (Hbn.)) but reasons for the increase were not definitely ascertained. The details of this particular phase of the study were published in another paper (Luckmann 1960).

The sap-loving Nitidulids, *Glischrochilus* spp., were more abundant where corn borers were most numerous and it is doubtful the dieldrin had any direct effect on this insect. However, the increase in number of European corn borers and other insects was not great enough to warrant additional control measures.

The dieldrin treatment appeared to have little adverse effect on many common predators. Adult and immature Coccinellids were quite abundant and this insect was observed on many occasions crawling through debris or walking on the soil and plants treated with sprays or granules of dieldrin. Immature and adult Nabids, Chrysopids, and Syrphids appeared not to be reduced by the treatment. The abundance of the very important egg predator, *Orius insidiosus*, (Say), appeared unchanged. The European corn borer parasite, *Lydella grisescens* R. D., was equally abundant in both treated and untreated areas. In untreated fields adjacent to the Japanese beetle control area, parasitism of corn borers by *L. grisescens* in 1954, 1955, and 1958 averaged 8.2%, 14.5%, and 20%, respectively. Parasitism of corn borers in treated fields averaged 5.5% in 1954, 19.3% in 1955, and 16.7% in 1958.

Some predators and parasites were eliminated by the treatment and the result is evidenced by the increase in population of some insects. Dryinids, common parasites of Cicadellids and Fulgorids, were noticeably absent in the treated areas. Leafhopper nymphs parasitized by Dryinid larvae were not regularly observed until the third season following treatment, and when 50% to 80% of the nymphs were parasitized in untreated bluegrass pastures only 13% were parasitized in treated pastures.

Carabids, particularly Carabid larvae, were absent for 2 years and the larvae were less abundant even the third year after treatment. Adults were more abundant than larvae in the treated area.

Early in the course of these investigations it was noted that earthworms were not eliminated in the treated fields. In 1958, samples were taken for the specific purpose of determining the number of earthworms in treated and untreated areas. The data obtained, table 3, show that during July, August, and September there were just as many earthworms in treated fields as in untreated fields. Several hours of observing while walking behind a plow on November 19, 1958, in the heptachlor area treated in 1956, in the dieldrin area of 1958, and adjacent untreated areas showed earthworms were about equally abundant and apparently of equal size in the three areas.

Since the insecticide did not appear to influence the number of earthworms present, samples of soil and of earthworms were collected and analyzed during April and May, 1959, to ascertain the amounts of dieldrin present in the worms and in the soil.

Analysis of the soil samples from treated areas showed that a considerable amount of insecticide was still present in the areas treated 4 to 5 years previously. Theoretically, a uniform application of 3 pounds of insecticide would have resulted in a maximum dosage of 1½ p.p.m. toxicant in the upper 6 2/3 inches of soil. The data indicate that 5 years after application the area treated in 1954 still retained one-third this amount. These persistent

Table 3.—The number of earthworms observed in 1958 in dieldrin-treated and untreated fields in the Japanese beetle control area at Sheldon, Illinois.

Year of Treatment	Crop	Amount of Dieldrin per Acre (lbs.)	No. of Earthworms per Square Foot to Depth of 9 Inches[a]			
			July 28	Aug. 28	Sept. 8	Average
1954	Corn	3	1.0	3.0	4.7	2.6
1954	Soybeans	3	1.2	3.8	8.9	4.7
1958	Corn	2	0.0	1.7	1.4	1.0
1958	Soybeans	2	1.7	10.0	14.3	8.7
No treatment	Corn	0	0.6	0.8	1.4	0.9
No treatment	Soybeans	0	0.4	4.1	10.6	5.0

[a] Sandy loam soil.

Table 4.—Amounts of dieldrin found in eight samples of earthworms and soil collected in the Japanese beetle control area at Sheldon, Illinois, during April and May, 1959.

Year Treated	Dosage of Dieldrin per Acre (lb.)	Interval (No. of Years from Treatment to Sampling)	P.P.M. Dieldrin[a]									
			Field 1		Field 2		Field 3		Field 4		Average	
			Soil	Earthworms	Soil	Earthworms	Soil	Earthworms	Soil	Earthworms	Soil	Earthworms
1954	3	5	0.73	0.30	0.45	0.36	0.48	0.34	0.35	0.25	0.50	0.31
1955	3	4	.51	.61	.80	.69	.58	.62	.89	.63	.70	.64
1957	2	2	.62	.30	.40	.25	.39	.20	.95	2.11	.59	.71
1958	2	1	.37	.31	1.82	2.41	.25	.19	.42	3.40	.72	1.60
Untreated	0	—	.00	.00	.07	.02	.00	.00	.00	.00	.00	.00

[a] Sandy loam soil.

residues of dieldrin help to explain its long-range effects on insects. There is considerable apparent variation in the data; for example, one soil sample showed an apparent dieldrin residue of 1.82 p.p.m. This may be due to overlap in application, non-uniform mixing of soil, or just plain sampling error.

The data presented in table 4 show that all earthworms taken in the treated area contained some dieldrin. In five of the 16 samples taken from treated areas, the earthworms contained more dieldrin than was found in the surrounding soil, which may indicate that under some conditions they can accumulate dieldrin in their bodies. Here again, the data are quite variable, which may be due to variations in species, soil type, or amounts and nature of food and organic matter. It may be that the amount of dieldrin to be found in earthworms is closely related to the amounts in the soil, but there seems to be an indication that at higher levels of intake earthworms may store proportionately larger amounts of dieldrin.

Farm Animals and Residues on Forage.—Shortly before treating began, arrangements were made with a farmer living in the area to place a cow on a pasture to be treated with granulated dieldrin. On April 12, 1954, a 5-acre, close-cropped bluegrass pasture belonging to the cooperator was treated with 30 pounds of 10% dieldrin granules per acre. Immediately after treatment the farmer turned the milk cow into the pasture and, at his own discretion, the cow's calf, twelve ewes (some with lambs), and many chickens. Between April 12 and April 30, the animals on the experimental pasture were observed every third day. Samples of milk obtained from the cow contained dieldrin. One-tenth p.p.m. toxicant was found after 1 day exposure and the amount increased up to 0.31 p.p.m. dieldrin on the 15th day of exposure. The amount of dieldrin in the milk showed a decline the 18th and 21st day after treatment but was not followed thereafter because the cow was removed from the pasture. All of the animals having access to the pasture appeared normal. On May 6, 1954, the plowed fields surrounding three sides of the pasture were sprayed by airplane with 3 pounds of dieldrin per acre. Some ewes became sick a few days after the spraying.

We should emphasize here the very great differences in the magnitude of residues on forage encountered after the granule and spray applications. For example, in the short grass pasture mentioned above, three samples of dried grass collected April 13, the day after the granule application showed 20, 53, and 21 p.p.m. of dieldrin, respectively, and averaged 31 p.p.m. Nearly 1 month later, 4 days after the aerial spraying of adjacent plowed fields, three samples taken from approximately the same spots showed residues of 553, 597, and 382 p.p.m., for an average of 511. These, of course, represented drift. No forage residues owing to direct spraying were available, because spraying was confined to plowed areas where the spray deposit would be disced into the soil and presumably no contamination of food or feed crops would occur.

On an adjacent farm also treated in 1954, six shorthorn heifers, two with calves, became ill while confined on a presumably untreated pasture. The milk from the heifer showing the most advanced symptoms of illness contained 1.14 p.p.m. of dieldrin. A diagram of the "untreated" pasture and the amounts of residue found on dried-grass samples in the pasture are illustrated in figure 1. The heifers were removed to dry lot and subsequently recovered.

Similarly on another farm ewes and lambs began to die while feeding on an "untreated" pasture. The milk from two ewes, each having lost twin lambs the day before, contained 2.2 and 8.7 p.p.m. of dieldrin, respectively. Subsequently both ewes died, and the one with 2.2 p.p.m. dieldrin in her milk was dissected. The amounts of toxicant found in some organs and tissues of her body are shown in table 5. The data leave little doubt as to cause of death.

These short paragraphs quickly explain the typical conditions existing in the treated area in 1954. Farm

Table 5.—Dieldrin residues in tissue samples taken from a ewe that died while feeding on a bluegrass pasture[a] exposed to drift from aerial spraying of 3 pounds of dieldrin per acre in the Japanese beetle control area at Sheldon, Illinois.

Type of Tissue	Toxicant	Toxicant Pound (p.p.m.)
Fat (check)	None	0.05[b]
Omental fat	Dieldrin	99.0
Renal fat	Dieldrin	130.0
Flesh (check)	None	0.09[b]
Flesh	Dieldrin	8.9
Liver (check)	None	0.04[b]
Liver	Dieldrin	13.0
Heart	Dieldrin	24.0

[a] Dosage was unknown; interval between general treatment of the area to sampling of tissue was 11 days.
[b] Apparent dieldrin.

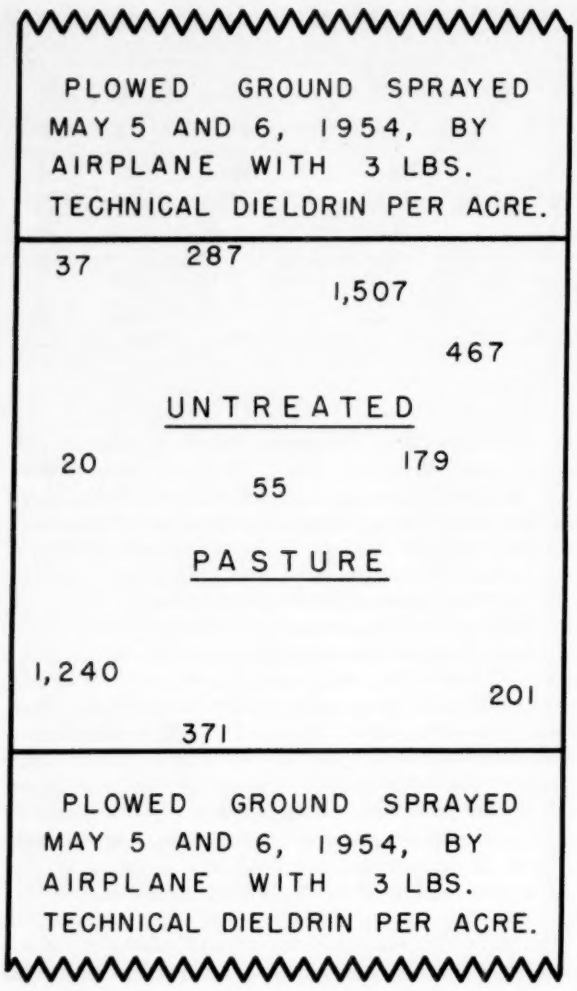

FIG. 1.—Diagram of a 35-acre untreated pasture near Sheldon, Illinois, in the Japanese beetle control area. Six shorthorn heifers became ill while on this pasture. Numbers indicate p.p.m. dieldrin found on dried grass samples on May 10, 1954, at the approximate locations shown.

animals exposed to pastures treated with dieldrin granules showed no evidence of adverse effects induced by the insecticide. On the other hand, while spraying plowed fields, drift regularly contaminated adjacent pastures and some animals eventually became sick and died. Sheep seemed to suffer most from the dieldrin spray treatment, and many of the sheep in the treated areas died within a week after the spray was applied. Seven beef animals were apparently poisoned, but only one animal died. On several farmsteads, household cats were practically exterminated.

Nearly all of the sick and dying livestock had been confined to pastures presumably untreated but in reality dangerously contaminated during the spraying operation; however, some animals that later became sick had been grazing for 2 to 4 weeks without ill effects on pastures treated with granules 18 to 24 days before spraying operations in adjacent fields began. As symptoms of poisoning developed while the animals were still on the so-called "clean or safe" pastures, considerable effort was devoted to determining the extent and degree of contamination.

In view of the hazards associated with the use of dieldrin sprays at these very high rates of application, only granulated materials were used in 1955 and later years. During 1955 and subsequent years, representative samples of all types of farm animals in the area were observed. In some instances, drastic measures were employed, such as staking and confining sheep to treated pastures immediately following application of granules. No illness of livestock attributable to dieldrin poisoning occurred from 1955 through 1958 when granules were used. In all instances, drinking water of the animals was covered during application of the granules. A summary of livestock conditions during the 5-year period is given in table 6.

To supplement the observations made on livestock, residue data were collected at intervals from several fields treated in 1955. The data presented in table 7 show the residues of dieldrin found in three fields of red clover when treated at intervals characterized by marked differences in wetness of plant surfaces on the morning of April 22, 1955. Field A was treated at 7 a.m. when the plants were very wet as a result of an extremely heavy dew that

Table 6.—A summary of the effects of dieldrin sprays and granules on farm animals in the Japanese beetle eradication area at Sheldon, Illinois.

TYPE OF FARM ANIMAL	EFFECTS OF DIELDRIN TREATMENT ON FARM ANIMALS IN THE JAPANESE BEETLE CONTROL AREA	
	Exposed to Drift from Spray Treatment in 1954	Exposed to Granules 1954–1958
	(3 lbs. tech. deildrin in 3 gals. solvent per acre)	(30 lbs. 10% dieldrin granules per acre.)
Dairy animals; cows[a] and calves.	No apparent ill effects.	No apparent ill effects.
Sheep, rams, ewes[b] and lambs.	Poisoning and death.	No apparent ill effects.
Beef animals; bulls,[c] heifers, calves.	Some poisoning and death of one adult animal.	No apparent ill effects.
Hogs, boars, sows[d] and pigs.	No apparent ill effects.	No apparent ill effects.
Poultry; week old to adult chickens.[e]	No apparent ill effects.	No apparent ill effects.
Dogs.[f]	No apparent ill effects.	No apparent ill effects.
Cats.[f]	Some poisoning and death.	Some poisoning and death.

[a] On bluegrass pastures, and legume-grass pastures.
[b] On bluegrass pastures.
[c] On bluegrass pastures.
[d] On bare-earth farm lots and alfalfa pastures.
[e] On bare-earth farm lots and alfalfa range.
[f] Around farmsteads.

Table 7.—Samples of red clover taken from pastures near Sheldon, Illinois, treated by airplane with 30 pounds of 10% dieldrin clay granules per acre at 7 a.m., 8 a.m., and 9:30 a.m. on April 22, 1955, when foliage was very wet, medium wet, and only slightly wet from dew moisture.

1955 Date Sampled	Interval After Treatment of Pastures	Final Height of Foliage (in.)	Treatment to Harvest (days)	Toxicant Found (p.p.m.)
Field A—Red clover, 7 a.m.				
Apr. 22	10 hours	7 to 9	1	19.5
Apr. 25	3 days	9 to 10	3	17.5
Apr. 28	6 days	9 to 11	6	4.5
May 5	13 days	11 to 12	13	1.7
May 12	20 days	12 to 15	20	0.40
May 19	27 days	15 to 18	27	0.38
Check	Pre-treatment			0.03
Field B—Red clover, 8 a.m.				
Apr. 22	9 hours	5 to 6	1	3.4
Apr. 28	6 days	9 to 12	6	1.9
May 5	13 days	12 to 14	13	0.48
May 12	20 days	16 to 20	20	0.45
May 19	27 days	24 to 26	27	0.18
Field C—Red clover, 9:30 a.m.				
Apr. 22	8 hours	8	1	0.85
Apr. 28	6 days	8 to 9	6	0.55
May 5	13 days	12 to 14	13	0.55
May 12	20 days	13 to 16	20	0.12
May 19	27 days	20 to 22	27	0.13

NOTE: 1.1 inches of rain fell on April 24 and 25.

morning. The granules readily adhered to the plants in this field, and analysis of the foilage showed an initial residue of 19.5 p.p.m. dieldrin. Conditions in this field were atypical. Some drying had occurred by 8 a.m., and clover plants treated at that time contained only 3.4 p.p.m. residue. The plants were only slightly wet at 9:30 a.m. and the amount of residue found adhering to the plants treated at this time was considerably less.

Residues of dieldrin were found in all of the meadow and pasture fields examined during April and May, 1955. The amount of dieldrin found shortly after treatment varied from less than 1 p.p.m. to slightly more than 19 p.p.m. Since much of the treating in 1954 and 1955 occurred after the foilage had made considerable growth, there is little doubt that all forage treated at that time carried detectable residues of dieldrin during the month following application. The treatments in 1956, 1957, and 1958 were made earlier in the year to minimize the possibility of forage contamination.

To determine the worst that could possibly happen to a milk supply, arrangements were made to pasture experimental animals in pasture A (table 7). In this test Holstein milk cows were placed in the pasture immediately

Table 8.—Samples of milk obtained from test Holstein cows feeding on a pasture treated with 30 pounds of 10% dieldrin clay granules per acre at 7 a.m. on April 22, 1955.

Animal No.	Date of Sample	Time After Treatment of Pasture	Exposure of Cow to Pasture (days)	Toxicant Found (p.p.m.) In Milk	Toxicant Found (p.p.m.) On Forage
Pre-test Checks	Apr. 18			0.11	0.03
Cow No. 1[a]	Apr. 22	10 hrs.	0	—	19.5
	Apr. 24	2 days	1	4.2	—
	Apr. 25	3 days	2	4.3	17.5
	Apr. 28	6 days	5	4.8	4.5
	May 5	13 days	12	2.5	1.7
	May 12	20 days	19	2.4	0.4
	May 19	27 days	26	2.8	0.38
Cow No. 2[b]	Apr. 26	4 days	0	0.13	6.5
	Apr. 27	5 days	1	1.4	5.3
	May 5	13 days	9	2.4	1.7
	May 12	20 days	16	1.0	0.4
	May 19	27 days	23	1.6	0.38
	May 23	31 days	27	1.6	
Cow No. 3[b]	May 5	13 days	9	2.2	
	May 23	31 days	27	1.3	

[a] Cow no. 1 placed on pasture 9 a.m. on April 23.
[b] Cows no. 2 and no. 3 placed on pasture 8 a.m. on April 26.

and 3 days following treatment. The recorded amounts of dieldrin found in the milk and on the forage the cows were eating are shown in table 8. Production records indicated no difference in the amount of milk produced after the cows were placed on the test pasture. Although residues ranging up to 19.5 p.p.m. of dieldrin on forage caused no harm to the cows, the milk from these animals was contaminated to the extent shown. It seems significant that delayed pasturing of only 3 days reduced milk contamination 50% even with the high initial residue present.

Considering the types of application and the many years of observation, it is apparent that livestock, such as cows, hogs, sheep, and chickens, feeding and living in pastures and farm lots were not harmed when dieldrin was applied in a granulated form at a dosage of 2 or 3 pounds of toxicant per acre. However, aerial sprays of the same amount of dieldrin, or even the drift from such sprays, did seriously affect some livestock, especially sheep.

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Alkoxyphenyl *N*-Methylcarbamates as Insecticides[1,2]

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Abstract

Twenty-four new alkoxyphenyl *N*-methylcarbamates were prepared and evaluated as insecticides against the house fly (*Musca domestica* L.), mosquito larva (*Culex quinquefasciatus* Say), salt-marsh caterpillar (*Estigmene acrea* (Drury)) and citrus red mite (*Panonychus citri* (McG.)). Many of the compounds were highly insecticidal and the *o*- and *m*-ethoxy-, *o*-isopropoxy-, *m*-propoxy, 3,4-methylenedioxy-, 2,5-dimethoxy-, and 3,5-dimethoxyphenyl *N*-methylcarbamates were most active. The latter two compounds showed the highest toxicity and knockdown activity to the house fly. The compounds of this class were markedly synergized by piperonyl butoxide.

The insecticidal properties of *N*,*N*-dimethyl- and *N*-methylcarbamic acid esters of various alcohols and phenols have been demonstrated by several investigators. Gysin (1954) investigated the properties of the *N*,*N*-dimethyl carbamates of heterocyclic alcohols and found the compounds pyrolan, or 1-phenyl-3-methylpyrazolyl *N*,*N*-dimethylcarbamate, and isolan, or 1-isopropyl-3-methylpyrazolyl *N*,*N*-dimethylcarbamate to be especially useful as insecticides. Meltzer (1956) described the insecticidal activity of S.17, or phenyl *N*,*N*-dimethylcarbamate. Kolbezen *et al.* (1954) studied the insecticidal properties of a number of substituted phenyl *N*-methylcarbamates of which the *o*- and *m*-alkylated phenyl derivatives were the most active insecticides; and Haynes, *et al.* (1957) described the insecticidal properties of Sevin® (α-naphthyl *N*-methylcarbamate) which is now in large scale commercial production. Within the past several years interest in these compounds has been heightened as it has become apparent that they represent an entirely new class of insecticides with unique insecticidal action.

This paper represents a continuation of the systematic evaluation of insecticidal properties of the *N*-methylcarbamic acid esters of phenols which was begun in a previous paper (Kolbezen *et al.* 1954), with the hope of correlating chemical structure and reactivity with insecticidal action. The present investigation is concerned with the properties of various alkoxyphenyl *N*-methylcarbamates.

Materials and Methods.—The carbamates evaluated in this study were all prepared by reacting the appropriate purified phenol with methyl isocyanate. Two general methods of preparation were used which are illustrated by the following examples: (a) *p*-ethoxyphenyl *N*-methylcarbamate was prepared by adding 1.3 ml. (1.34 gm. or 0.023 moles) of methyl isocyanate (Colucci 1945) to 10 ml. anhydrous diethyl ether containing 2.76 gm. *p*-ethoxyphenol (0.02 moles) and 3 drops triethylamine, in a small glass stoppered flask. After standing for 1 week at room temperature, the solvent was removed and the product was obtained as 3.0 gm. of white needles (yield 77%). The product was recrystallized from Skellysolve B and obtained as long white needles, m.p. 95° to 96°. In method (b) *o*-*n*-propoxyphenyl *N*-methylcarbamate was prepared by adding 3 ml. methyl isocyanate to 3.02 gm. *o*-*n*-propoxyphenol, containing 2 drops of triethylamine, in a pressure bottle. The mixture was heated on the steam bath for 4 hours, the excess methyl isocyanate removed and the crude crystals obtained were recrystallized from Skellysolve B to give white needles, m.p. 68° to 69°. The yield was virtually quantitative.

Some of the carbamates were induced to crystallize with difficulty, *i.e.*, only after standing for several weeks, upon trituration with Skellysolve B, or after washing with 5% sodium hydroxide to remove the unreacted phenol. Compounds VI, X, XIV, and XXII crystallized only after distillation at high vacuum. Compounds VIII, X, XI, XIII, and XIV were recrystallized from Skellysolve A, XV, and XVIII from ethylacetate, XXIII from toluene, and the remainder from Skellysolve B. The properties and analytical values for the compounds prepared are given in table 1.

The longer chain mono-alkoxyphenols which were unavailable commercially were prepared by the method of Klarmann *et al.* (1931, 1932). The dialkoxyphenols were prepared by a variety of specific reactions as follows: 3,5-Dimethoxyphenol and 3,5-diethoxyphenol were prepared according to Pratt & Robinson (1924). 2,4-Dimethoxyphenol and 2,5-dimethoxyphenol were prepared by oxidizing the Grignard intermediates prepared from 2,4-dimethoxybromobenzene and 2,5-dimethoxybromobenzene, respectively, according to Gilman & Van Ess (1939). 2,3-Dimethoxyphenol was prepared from pyrogallol according to Baker & Smith (1931). 3,4-Dimethoxyphenol was prepared by the peracetic acid oxidation of 3,4-dimethoxybenzaldehyde according to Böeseken & Greup (1939). 3,4,5-Trimethoxyphenol was prepared by the procedure described by Chapman *et al.* (1927). Orcinol monomethyl ether (3-methyl-5-methoxyphenol) was prepared as described by Hiers & Hager (1929). The remaining phenols were obtained from commercial sources.

The evaluations in insect toxicity were made exactly as described by Fukuto *et al.* (1959) and the anticholinesterase activity as described by Kolbezen *et al.* (1954).

Discussion of Results.—The relative activity of the various alkoxyphenyl *N*-methylcarbamates to several species of insects is given in table 2 in comparison with Sevin (1-naphthyl *N*-methylcarbamate) and *m*-isopropylphenyl *N*-methylcarbamate.[3] The information presented indicates something of the surprisingly specific action of this class of insecticides. The *m*-isopropylphenyl *N*-methylcarbamate like many of the other alkylphenyl *N*-methylcarbamates studied (see Kolbezen *et al.* 1954) is extremely toxic to the larvae of the salt-marsh caterpillar, *Estigmene acrea* (Drury), and produced violent convulsions and profuse regurgitation and defecation of the larvae after a few minutes exposure to residues as low as 6 micrograms per sq. cm. This compound is also highly

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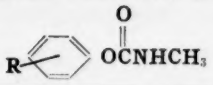
[3] Samples furnished as Hercules Powder Company 5727 and Union Carbide Chemicals Company 10,854.

Table 1.—Physical constants of substituted phenyl *N*-methylcarbamates.



R	M.P. °C.	Molecular Formula	Analysis	
			Calculated (%)	Found (%)
I. *o*-CH_3O	88–90	$C_9H_{11}NO_3$	C 59.71 H 6.12	C 60.24 H 6.58
II. *m*-CH_3O	71–2	$C_9H_{11}NO_3$	C 59.71 H 6.12	C 59.83 H 6.52
III. *p*-CH_3O	89–91	$C_9H_{11}NO_3$	C 59.71 H 6.12	C 59.52 H 6.44
IV. *o*-C_2H_5O	79.5–80.5	$C_{10}H_{13}NO_3$	C 61.52 H 6.71	C 61.73 H 7.14
V. *m*-C_2H_5O	55–6	$C_{10}H_{13}NO_3$	C 61.52 H 6.71	C 62.14 H 7.42
VI. *p*-C_2H_5O	95–6	$C_{10}H_{13}NO_3$	C 61.52 H 6.71	C 62.10 H 7.02
VII. *o*-*n*-C_3H_7O	68–9	$C_{11}H_{15}NO_3$	C 63.14 H 7.23	C 63.00 H 7.07
VIII. *m*-*n*-C_3H_7O	58.5–9.5	$C_{11}H_{15}NO_3$	C 63.14 H 7.23	C 63.53 H 7.76
IX. *o*-iso-C_3H_7O	77–9	$C_{11}H_{15}NO_3$	C 63.14 H 7.23	C 62.50 H 7.59
X. *m*-iso-C_3H_7O	56–8	$C_{11}H_{15}NO_3$	C 63.14 H 7.23	C 63.39 H 7.75
XI. *o*-*n*-C_4H_9O	36–9	$C_{12}H_{17}NO_3$	C 64.55 H 7.67	C 64.30 H 7.50
XII. *m*-*n*-C_4H_9O	54.5–5.5	$C_{12}H_{17}NO_3$	C 64.55 H 7.67	C 64.66 H 8.18
XIII. *m*-sec-C_4H_9O	52–4	$C_{12}H_{17}NO_3$	C 64.55 H 7.67	C 65.05 H 8.09
XIV. *m*-iso-C_4H_9O	50–2	$C_{12}H_{17}NO_3$	C 64.55 H 7.67	C 64.89 H 8.43
XV. 2,3-diCH_3O	103–5	$C_{10}H_{13}NO_4$	C 56.86 H 6.20	C 56.78 H 6.44
XVI. 2,4-diCH_3O	95–6	$C_{10}H_{13}NO_4$	C 56.86 H 6.20	C 56.90 H 6.46
XVII. 2,5-diCH_3O	96–8	$C_{10}H_{13}NO_4$	C 56.86 H 6.20	C 56.75 H 6.48
XVIII. 2,6-diCH_3O	136–7	$C_{10}H_{13}NO_4$	C 56.86 H 6.20	C 56.80 H 6.72
XIX. 3,4-diCH_3O	99–100.5	$C_{10}H_{13}NO_4$	C 56.86 H 6.20	C 57.36 H 6.31
XX. 3,5-diCH_3O	76.5–77.5	$C_{10}H_{13}NO_4$	C 56.86 H 6.20	C 57.09 H 6.45
XXI. 3,4-methylenedioxy	104–5	$C_9H_9NO_4$	C 55.38 H 4.64	C 55.85 H 4.76
XXII. 3,5-diC_2H_5O	69.5–71.5	$C_{12}H_{17}NO_4$	C 60.24 H 7.16	C 61.77 H 7.48
XXIII. 3,4,5-triCH_3O	104–8	$C_{11}H_{15}NO_5$	C 54.76 H 6.26	C 54.88 H 6.59
XXIV. 3-methyl-5-methoxy	b.p. 133°/0.2 mm.	$C_{10}H_{13}NO_3$	C 62.03 H 6.71	C 62.98 H 7.04

Table 2.—Toxicity data for substituted phenyl *N*-methylcarbamates.

R (see Table 1)	I_{50} M Fly ChE	*Musca domestica* LD_{50} γ per Gm.	*Culex 5-fasciatus* Approximate LC_{50} P.P.M.	*Estigmene acrea* Approximate LC_{50} Mg. per Leaf Disc
I. *o*-CH_3O	3.7×10^{-5}	92.5	>10	>10
II. *m*-CH_3O	2.2×10^{-5}	90.0	10	3–10
III. *p*-CH_3O	8.0×10^{-5}	>500	20	>10
IV. *o*-C_2H_5O	1.6×10^{-5}	55	2	> 3
V. *m*-C_2H_5O	6.0×10^{-6}	75	3	0.3–1
VI. *p*-C_2H_5O	7.0×10^{-5}	>500	>10	>10
VII. *o*-*n*-C_3H_7O	8.7×10^{-6}	105	2	> 1
VIII. *m*-*n*-C_3H_7O	1.6×10^{-5}	95	0.9	1–3
IX. *o*-iso-C_3H_7O	6.9×10^{-7}	25.5	0.3	0.3–1
X. *m*-iso-C_3H_7O	9.2×10^{-6}	180	3	3–10
XI. *o*-*n*-C_4H_9O	1.2×10^{-5}	175	5	1–3
XII. *m*-*n*-C_4H_9O	9.4×10^{-6}	280	0.3	3–10
XIII. *m*-sec-C_4H_9O	7.0×10^{-6}	220	1.5	3–10
XIV. *m*-iso-C_4H_9O	2.0×10^{-5}	>500	2	3–10
XV. 2,3-diCH_3O	1.4×10^{-5}	>500	>10	3–10
XVI. 2,4-diCH_3O	2.8×10^{-5}	155	>10	>10
XVII. 2,5-diCH_3O	1.3×10^{-5}	13.0	>10	0.3–1
XVIII. 2,6-diCH_3O	2.1×10^{-3}	>500	>10	>10
XIX. 3,4-diCH_3O	1.9×10^{-5}	400	>10	3–10
XX. 3,5-diCH_3O	8.0×10^{-6}	11.0	>10	0.3–1
XXI. 3,4-methylenedioxy	1.3×10^{-4}	17.5	>10	0.3–1
XXII. 3,5-diC_2H_5O	1.1×10^{-5}	40.0	>10	> 1
XXIII. 3,4,5-triCH_3O	6.5×10^{-6}	32.0	>10	> 1
XXIV. 3-methyl-5-methoxy	1.7×10^{-5}	37.5	6	0.3–1
XXV. 3-isopropyl	3.4×10^{-7}	90	0.03	0.3–1
XXVI. Sevin	9.0×10^{-7}	>500	0.5	1–3

toxic to the larva of the southern house mosquito, *Culex quinquefasciatus* Say, but is only of moderate activity against the house fly, *Musca domestica* L. Sevin is somewhat less active against both *E. acrea* and the mosquito and is of low activity to the house fly. The mono-alkoxyphenyl *N*-methylcarbamates such as the *o*-ethyl (IV) *m*-ethyl (V) and *o*-isopropyl (IX) were surprisingly active against the house fly and the 2,5- and 3,5-dimethoxyphenyl *N*-methylcarbamates (XVII and XX) were highly toxic to this insect with LD_{50} values of 0.26 and 0.22 micrograms per female fly as compared with 0.58 micrograms for malathion. However, only V, IX, XVII, XX, and XXI of the alkoxyphenyl *N*-methylcarbamates approached the activity of *m*-isopropylphenyl *N*-methylcarbamate to *E. acrea* larvae, and none of the alkoxyphenyl *N*-methylcarbamates were as active against *Culex* larvae. In experiments to determine the acaricidal activities of residues of these compounds against the adult female citrus red mite, *Panonychus citri* (McG.), the only compounds showing appreciable activity under the test conditions were II, VII, IX, and XI which had 24-hour LC_{50} values of about 0.1% while that of all the other compounds was greater than 1%.

Relation of Structure to Activity.—In a previous paper from this laboratory (Kolbezen *et al.* 1954) the insecticidal activity of various substituted phenyl *N*-methylcarbamates was discussed in terms of their action as competitive inhibitors of cholinesterase (ChE), a logical extension of the large amount of data available from studies of the pharmacological action of the closely related physostigmine and prostigmine (Stedman 1926). The requirements for toxic action under this concept are (a) structural complementariness to acetyl choline (ACh), the normal substrate of ChE, (b) sufficient stability to the hydrolytic action of ChE to permit the compound to act as a competitive blocking agent for ChE rather than as a substrate, and (c) proper lipoid solubility and absence of a permanent electrical charge which permit penetration of the lipoid sheath surrounding the insect nerve (Winton *et al.* 1958).

The importance of position-isomerism in the dimethylaminophenyl *N*-methylcarbamate methiodides was recognized by Stedman (1926) who found the *m*-substituted compound much more active than the *o*- and *p*-isomers. In the variously substituted compounds studied by Kolbezen *et al.* (1954), the *m*-substituted compounds were most active, closely followed by the *o*-isomers, while the *p*-isomers were much less active. This was considered to represent structural conformity to the enzyme surface, since the *m*- and *o*-compounds most closely correspond in spatial dimensions to acetyl choline, as can be seen clearly with molecular models. In accounting for the reactivity, or conversely the stability, of the substituted phenyl *N*-methylcarbamates, the quantitative measurement of the relative electron-withdrawing or donating properties of the various substituents, as expressed by Hammett's sigma values, was found to give a close approximation to the relative activity as ChE inhibitors. The *m*- and *p*-methoxyphenyl *N*-methylcarbamates and *m*- and *p*-ethoxyphenyl *N*-methylcarbamates closely fit the relationship developed by Kolbezen *et al.* (1954) between the $-\log K_i$ for fly cholinesterase *vs.* Hammett's sigma values for the electron-withdrawing effects of the substituents as shown in their figure 2. Unfortunately it has been impossible to develop representative sigma values for *o*-substituents because of the well known "ortho effect" of polar, steric, and resonance interaction between the substituent and the reaction center, the carbamoyl-phenol bond (Taft 1956).

With the multi-substituted alkoxyphenyl *N*-methyl-

carbamates, the situation is more complex. A beginning has been made in the application of sigma values to multiple substituents on the aromatic nucleus where the values are generally additive unless they are adjacent, where steric inhibition of resonance or hydrogen bonding may occur (Jaffe 1953). However, pronounced steric effects of the position-isomers in regard to their reaction with cholinesterase as shown by Kolbezen *et al.* (1954) makes quantitative interpretations of this type most difficult at our present state of knowledge. The data in table 2 show that the di-methoxyphenyl *N*-methylcarbamates have a remarkable uniformity in I_{50} values for fly cholinesterase, with the exception of the 2,6-compound (XVIII) which is about 0.01 as active as the others. Compound XVIII must owe its inactivity to its two *ortho*substituents which provide a high degree of ortho-interaction and possibly steric hindrance to combination with cholinesterase. The reason for the inactivity of the methylene dioxyphenyl *N*-methylcarbamate (XXI) as an inhibitor and yet its high toxicity to the house fly is mystifying. Perhaps this compound serves as its own synergist as discussed later.

With regard to the correlation of structure and anticholinesterase activity to insecticidal action it is clear from the data in table 2 that some very complex relationships exist. In general the most active anticholinesterase compounds such as V, VII, IX, XX, and XXIII were highly toxic. However, from the data presented below on synergism it is evident that the rate of detoxication in the insect body varies greatly with the position and nature of the substituent(s) and that insect toxicity is directly associated with this factor. Until such specific detoxication systems are more carefully evaluated no further generalizations can be made, but it appears to the authors that the remarkable specificity shown by these carbamate insecticides must be associated with the presence or absence of such specific detoxifying enzymes.

SYNERGISM OF CARBAMATES WITH PIPERONYL BUTOXIDE.—The marked synergistic effects of pyrethrin synergists such as piperonyl butoxide, sesamex (Sesoxane), sulfoxide, *n*-propyl isome and sesame oil to carbamates was demonstrsted by Moorefield (1958). With piperonyl butoxide used at a ratio of 5 parts to 1 part of carbamate he obtained activation (measured at the LD_{50} level to house flies) of about 33 times for Sevin, 22 times for *m*-tertbutylphenyl *N*-methylcarbamate, and 10 times for Isolan and Pyrolan. Similar results have been reported by Eldefrawi *et al.* (1959) and Fuchs *et al.* (1959).

Some of the alkoxyphenyl *N*-methylcarbamates studied in this paper have been evaluated for synergism with piperonyl butoxide at the 5:1 ratio with the results shown in table 3. Very appreciable synergism to house flies was shown for all the compounds evaluated and it appears that the degree of synergism found is inversely correlated with the innate toxicity of the compound. This is clearly demonstrated with the 3,4-dimethoxyphenyl *N*-methylcarbamate (XIX) which was only weakly toxic and yet had an activation ratio of 33 times, as compared with 3,5-dimethoxyphenyl *N*-methylcarbamate (XX) which was the most active compound studied and to the house fly had an activation ratio of only 2.5 times. This information has important implications in judging the relation of structure to activity of the carbamate compounds. It is apparent from table 2 that most of the series studied, excluding the *p*-substituted compounds and the 2,6-disubstituted analogue (XVIII), had I_{50} values for fly cholinesterase ranging between 7×10^{-6} and 3×10^{-5}. These should therefore be expected to be of reasonably comparable toxicity to the house fly as measured by the LD_{50} values. However, the toxicity data in table 2 show a range of LD_{50} values from 11 to >500 micrograms per gram of fly body weight. There thus appears to be no pronounced correlation between enzyme-inhibition and toxicity until the toxicity is measured in the presence of a synergist such as piperonyl butoxide.

Table 3.—Synergism of several carbamates with piperonyl butoxide (P.B.).

SUBSTITUTED PHENYL *N*-METHYLCARBAMATE	*Musca domestica* LD_{50} γ PER GM.		RATIO OF SYNERGISM
	A (alone)	B (1:5 P.B.)	A/B
m-ethoxy-	75	9.5	7.9
o-isopropoxy-	25.5	7.0	3.6
m-isopropoxy-	180	19.5	9.2
3,4-dimethoxy-	400	12.0	33
3,5-dimethoxy-	11	4.4	2.5

The available information on the action of piperonyl butoxide as a synergist for pyrethrins, DDT against resistant house flies, carbamates, and phosphates, strongly suggests that this and similar synergists act as inhibitors of detoxication mechanisms (Metcalf 1955, Moorefield 1958). These detoxication mechanisms are most probably relatively nonspecific esterase systems which rapidly degrade the toxic insecticidal compounds before they reach the site of action. The data recorded here are in complete agreement with this hypothesis and as shown in table 3, with simultaneous application of piperonyl butoxide the LD_{50} values for the compounds studied are reduced from a range of about 40-fold to about 4-fold. A more detailed appraisal of this subject and a discussion of its broad implications in insect toxicology will be presented in subsequent papers from this laboratory. It should be mentioned that the structure of the 3,4-methylenedioxyphenyl *N*-methylcarbamate (XXI) is closely related to that of synergists of the piperonyl butoxide type and this may account for its apparently anomalous behavior.

In conclusion, it may be stated that the alkoxyphenyl *N*-methylcarbamates have very interesting insecticidal properties. It is apparent that the present investigation has by no means exhausted the possibilities of this group, as 2-isopropoxy-5-methoxyphenyl *N*-methylcarbamate m.p. 96° to 98° has recently been prepared and found to have an LD_{50} value to the house fly of 6.5 micrograms per gram which makes it considerably more active than XX. Work is continuing along these lines and will be reported in a subsequent paper.

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Residues in Vegetable Crops Following Soil Applications of Insecticides[1]

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ABSTRACT

Of 15 vegetables grown in soil treated with 10 pounds of chlordane per acre, only sweet potato, carrot, and rutabaga had residues in excess of 0.3 p.p.m. Of 12 grown in soil treated with 20 pounds of DDT per acre, only potato, bell pepper, and table beet had residues. Potatoes contained 0.5 p.p.m. Of eight crops grown in soil treated with 42 pounds of ethylene dibromide per acre, table beet, onion, turnip, sugar beet, and lima bean straw showed residues of 9 to 76 p.p.m. Radishes grown in soil treated with 4 pounds of aldrin per acre contained no aldrin but 0.24 p.p.m. of dieldrin; no residue was detected from the same dosage of dieldrin. Sugar beets and table beets grown in soil treated with 3 pounds of toxaphene per acre showed 0.4 p.p.m. or less.

Studies of residues on various vegetable crops grown in soil treated with insecticides were conducted at Whittier, Calif. In 1957–58 the major objective was to clear for continued use certain chlordane, DDT, and toxaphene treatments that were well established before tolerances were set but for which there were no adequate supporting data. Data needed to extend the use of ethylene dibromide and certain other soil insecticides for the protection of additional crops were also obtained.

Erwin *et al.* (1956) and Brett & Bowery (1958) have previously reported on residues in vegetables resulting from foliage and soil applications.

METHOD OF APPLICATION.—The tests were made on small plots of sandy loam soil not previously treated with insecticides. In 1957 single plots 24 by 43 feet were used, but in 1958 each treatment was applied to three plots, each 35 feet square. To avoid contamination the treated plots were separated by 5-foot alleys and the check plots were located at the end or along side of the experimental area. The insecticides were applied in 100 gallons of emulsion per acre to the soil surface with hand or power sprayers, and immediately mixed with the soil by disking 6 to 7 inches deep. Toxaphene at 3 pounds per acre, as used for cutworm control, was incorporated with the soil before planting, and also sprayed on the surface during the seedling stage. The seed bed was prepared and the crops were planted 2 to 49 days after treatment. The crops were irrigated at 2-week intervals and dusted with 2% parathion or 4% malathion when needed for insect control.

Other plots were used for obtaining data on bromine residues in crops following injections of ethylene dibromide into the soil 7 to 8 inches deep, at 12-inch spacings, at the rate of 42 pounds per acre, which is recommended for wireworm control.

SAMPLING AND METHOD OF ANALYSIS.—Samples were

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Table 1.—Residues in crops grown in soils treated with insecticides, 1957–58.

Insecticide (lb./a.)	Date Applied	Crop	Weeks Between Treatment and Harvest	Residue (p.p.m.)	
Aldrin 4	April 25, 1957	Lima bean (Fordhook) shelled, green	16		—[a]
		Sweet potato (Puerto Rico)	19	Aldrin	0.03
				Dieldrin	.03
	March 3, 1958	Radish (Scarlet Globe)	5	Aldrin	[b]
				Dieldrin	0.24
Chlordane 10	April 25, 1957	Sugar beet (U. S. 56) roots	24	Chlordane	0.04
	March 5, 1958	Carrot (Longtype 48)	16		1.51
		Broccoli (Calabrase)	17		—[b]
		Turnip (Purple Top) roots and tops	12		0.16
		Tomato (Pearson)	18		0.01
		Eggplant (N. Y. Improved)	20		—[b]
		Cucumber (Marketer)	14		0.08
		Bell pepper (Calif. Wonder)	17		—[b]
		Lettuce (Great Lake)	15		0.04
		Rutabaga (Purple Top)	15		0.50
		Sweet potato (Puerto Rico)	20		0.43
		Cauliflower (Snowball)	14		—[b]
		Cabbage (Early Jersey)	13		—[b]
		Cantaloupe (Hales Best)	18		0.08
		Collard (Georgia)	16		—[b]
DDT 10	April 25, 1957	Sweet potato (Puerto Rico)	19		0.2
		Lima bean (Fordhook) green, shelled	16		—[b]
DDT 20	March 5, 1958	Cauliflower (Snowball)	14		—[b]
		Collard (Georgia)	16		—[b]
		Broccoli (Calabrase)	17		—[b]
		Turnip (Purple Top) roots and tops	12		—[b]
		Eggplant (N. Y. Improved)	20		—[b]
		Kale (Dwarf Green Kurl)	16		—[b]
		Brussels sprouts (Catskill Select)	18		—[b]
		Potato (White Rose)	18		0.5
		Table beet (Early Wonder) roots and tops	13		0.5
		Rutabaga (Purple Top)	15		—[b]
		Bell pepper (Calif. Wonder)	17		0.2
		Strawberry (Lassen)	16		—[b]
Dieldrin 4	April 25, 1957	Sugar beet (U. S. 56) roots	19		0.11
		Radish (Scarlet Globe)	5		—[b]
Ethylene dibromide 42	April 5, 1957	Lima bean (Ventura) straw, dried	20	Bromine	17.8
	April 20, 1957		19		76.0
	May 5, 1957		16		28.0
	May 14, 1957	Lettuce (Great Lake)	14		14.9
		Table beet (Early Wonder) roots	10		10.4
		Sugar beet (U. S. 56):			
		tops	18		18.3
		roots	16		30.4
		Spinach (Giant Noble)	8		11.1
		Onion (Sweet Spanish)	16		9.1
		Turnip (Purple Top) roots	8		10.8
Ethylene dibromide 60	June 21, 1957	Blackeye bean, shelled	14		—[b]
Toxaphene					
Preplanting treatment	April 25, 1957	Sugar beet (U. S. 56) roots	18		—[b]
		Table beet (Early Wonder) roots	9		—[b]
		Potato (White Rose)	14		0.3
On surface, seedling stage	May 23, 1957	Potato (White Rose)	11		0.3
		Table beet (Early Wonder) roots	5		—[b]
		Sugar beet (U. S. 56) roots	15		0.3
	March 3, 1958	Radish (Scarlet Globe)	5		0.4

[a] No organic chlorine detected by Philips sodium reduction method.
[b] Below detectable level.

collected from the plots at random. The sample size varied from 2 pounds for radishes to 30 pounds for sugar beets. Such crops as celery, cabbage, lettuce, and cauliflower received a small amount of trimming. All root crops were washed with a spray of water. The trimming and washing were done in a manner similar to the commercial practice in this area. At the laboratory the samples were stored in a freezer until they could be processed. The frozen samples were finely ground and mixed, and representative subsamples of 300 to 500 grams were extracted with the appropriate solvent at the rate of 1 gram of plant material per 2 ml. of solvent by tumbling for 1 hour at 50 r.p.m. The extracts were stored in a refrigerator until the analyses were completed.

The methods used for determining the various residues were as follows: DDT, toxaphene, and methoxychlor—Phillips & DeBenedictis (1954); chlordane—Ordas *et al.* (1956); parathion—Averall & Norris (1948); bromide—Schrader *et al.* (1942); aldrin and dieldrin—O'Donnell *et al.* (1955, 1956). The samples from the aldrin-treated plots were analyzed for both aldrin and dieldrin, since there may be a conversion of aldrin into dieldrin in plants (Gannon & Decker 1958). The two materials were separated from each other with a magnesia-Hyflosupercel chromatographic column.

RESULTS.—The results of these analyses are presented in table 1. No residue of chlordane was found in collards, cauliflower, bell peppers, cabbage, eggplant, or broccoli, and less than 0.1 p.p.m. in tomatoes, sugar-beet roots, cantaloupes, lettuce, and cucumbers. The sweet potato, rutabaga, and carrot residues were all in excess of the Federal tolerance of 0.3 p.p.m.

No residue of DDT was found in 9 of the 12 crops grown in soil treated with 20 pounds per acre. Those in table beets and bell peppers were below the 7 p.p.m. tolerance. Potatoes also contained 0.5 p.p.m. of DDT, but since no residue is permitted in or on potatoes, DDT should not be applied in soil to be planted to this crop. No residue was found in green shelled lima beans and only 0.2 p.p.m. in sweet potatoes grown in soil treated with 10 pounds of DDT per acre.

Of the vegetables grown in soil fumigated with ethylene dibromide, three root crops (table beets, onions, and turnips) contained 9 to 11 p.p.m. of inorganic bromine. No tolerance resulting from soil fumigation has been established for these vegetables, but tolerances have been established for other root crops, including 50 p.p.m. for sweetpotatoes and 75 p.p.m. for carrots and parsnips. The tops and roots of sugar beets were analyzed separately. In practice lima bean vines are cut, field-dried, and threshed to harvest the dry shelled beans, and the dried vines may be used for cattle feed.

Four vegetables were grown in soil that received aldrin or dieldrin. Radishes from the aldrin-treated soil were found to contrain no aldrin, but 0.24 p.p.m. of dieldrin. The conversion of aldrin to dieldrin in plants is known to occur. Radishes from dieldrin-treated soil contained no detectable dieldrin. The established tolerances of aldrin and dieldrin in radishes are 0.25 and 0.10 p.p.m., respectively.

Residues of toxaphene in potatoes, table beets, sugarbeet roots and tops, and radishes grown in soil treated with toxaphene were less than 0.4 p.p.m.

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Systemic Insecticides Applied to Cut Seed Pieces and to Soil at Planting Time to Control Potato Insects[1]

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ABSTRACT

The systemic insecticides phorate, formerly designated as Thimet® (*O,O*-diethyl *S*-(ethylthio)methyl phosphorodithioate), Di-Syston® (*O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorodithioate) and demeton were evaluated in a series of field trials for control of the green peach aphid, *Myzus persicae* (Sulz.), the leafhopper, *Empoasca filamenta* DeL., and the tobacco flea beetle, *Epitrix hirtipennis* (Melsh.) on potatoes. The three insecticides were applied on activated charcoal to cut potato seed pieces. Granulated phorate and Di-Syston were applied to the soil with the seed pieces or with fertilizer at planting time. Demeton was also applied to cut seed pieces as a 0.75% emulsion dip. The charcoal seed piece treatments with phorate 44% and Di-Syston 50% at 2.6 ounces of formulated material per 100 pounds of seed pieces provided good control of the green peach aphid for 77 days. Applications of 2% phorate granules or 2% Di-Syston granules at 100 pounds per acre in the seed furrow controlled the green peach aphid for 86 days. Di-Syston generally gave longer aphid control than phorate. The demeton dip was more effective than the charcoal formulation in controlling the green peach aphid but in general control was poor.

Seed piece treatments with carbon formulations of phorate and Di-Syston were not as effective as applications of granules in controlling *filamenta*. Applications of phorate or Di-Syston granules gave excellent leafhopper control for 97 to 100 days. The two materials appeared to be about equal in effectiveness. Demeton failed to control this leafhopper.

The tobacco flea beetle was better controlled with seed piece treatments of charcoal formulated phorate and Di-Syston than with granules. Phorate resulted in slightly better control than Di-Syston.

Charcoal formulations of phorate and Di-Syston, especially at dosages of 2.6 ounces per 100 pounds of seed, greatly retarded plant emergence and in some instances reduced stand. Injury was especially severe on potatoes grown in peat soil. Applications of granules had less effect on delaying plant emergence although when applied in the seed furrow some slight reductions in stand resulted. The placing of the insecticide granules in fertilizer bands away from the seed pieces had little or no adverse effect on emergence and plant growth. Treating the seed pieces with demeton both as powders and as a dip appeared to stimulate plant growth. Plants from demeton-treated seed pieces emerged ahead of all other treatments.

Residue studies with phorate indicate that applications of this material to seed pieces or to the soil at planting time leave no detectable residues on the tubers 74 or 91 days later.

The development of insecticides that become distributed systemically within plants offers intriguing possibilities in insect control especially if these materials can be applied to the seed or to the soil at planting time to provide protection beginning with the earliest plant emergence. Much experimental work has been conducted with systemic insecticides applied to seeds and to the soil at planting time to protect seedling plants from insect and spider mite attacks. Most of the work has involved such crops as alfalfa, cotton, crucifers, and sugar beets: Reynolds *et al.* (1957), Parencia *et al.* (1957), Andres *et al.* (1959), Hopkins *et al.* (1959). Relatively little information has been published regarding the application of systemic insecticides to cut seed pieces or to the soil to control insect pests of potato. Klostermeyer (1953), in greenhouse studies, treated seed potatoes by dipping them in various dilutions of schradan and demeton. He reported kill of aphids 23 days after treatment but little mortality 39 days later. Demeton was more toxic to the aphids than schradan and its effects lasted for a longer time. The higher concentrations of both materials (0.25%) reduced plant growth. Hoyman (1958) studied the effect of phorate on incidence of potato virus-Y and purple-top wilt in Red Pontiac potatoes. He reported that phorate formulated at the rate of $3\frac{1}{3}$ pounds actual in 250 pounds of 12-12-12 granulated fertilizer and applied in bands at this rate per acre at planting time was not effective in reducing the incidence of virus-Y transmitted by the green peach aphid, *Myzus persicae* (Sulz.), but there was a significant decrease in the incidence of purple-top wilt transmitted by the six-spotted leafhopper, *Macrosteles fascifrons* (Stål). Hoyman's data were concerned entirely with the incidence of disease in the treated and untreated plots and did not include information on insect control *per se*.

To study the effects on aphids, leafhoppers and flea beetles of systemic insecticides applied to cut seed pieces and to the soil at planting time, a series of field trials on potatoes was conducted at Shafter and at Davis in 1957 and at Davis and Stockton, California in 1958.

METHODS AND MATERIALS.—The seed pieces used in the experiments were of the White Rose variety and were cut to weigh approximately 1.5 to 2 ounces each. The insecticides tested included phorate, demeton, and Di-Syston® (*O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorodithioate). Charcoal formulations (44% phorate, 50% demeton, and 50% Di-Syston) were each applied to the cut seed pieces at dosages of 1.3, 2.0, and 2.6 ounces per 100 pounds of seed. The seed pieces were treated in a rotary cement mixer and were tumbled with the dry powders for 2 minutes. Check seed pieces were tumbled for 2 minutes without an insecticide.

In the 1957 experiments demeton was also applied to cut seed pieces as a 0.75% emulsion dip by immersing the seed pieces in the emulsion for 2 minutes. Granulated formulations containing 2% phorate and 2% or 2.5% Di-Syston were applied with a special fertilizer applicator attached to an assist feed potato planter. The granules were dropped through the seed chute with the seed pieces at rates of 100 pounds of granules per acre. In 1958, the granulated insecticides were placed with the fertilizer in bands on either side of the seed pieces. Each treatment was replicated four times in a randomized block design in each experiment. The individual plots at Shafter and at

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Stockton were four rows wide (row spacing 32 inches) and 75 feet long. In the Davis plots the rows were 37.5 feet long in 1957 and 50 feet long in 1958. The seed pieces were planted 1 foot apart in the rows in all experiments.

Aphid counts were made 63, 70, 77, and 86 days after planting at Shafter. On each sampling date, 25 basal, compound leaves were selected at random from plants in the two center rows of each replication (100 leaves per treatment). Winged aphids, apterae and nymphs were recorded separately. In presenting the data, however, only the apterae and nymphs are included, and these are combined. To evaluate control of flea beetles at Davis, in 1957, the numbers of feeding scars in leaves in the various treatments were recorded. These counts were made 42 days after planting. Forty leaves were examined per treatment, 10 per replication from the two center rows of each plot.

Leafhopper counts in the Davis experiments were begun 77 days after planting in 1957 and 73 days after planting in 1958. Ten leaves were selected at random from each of the four replicates of each treatment on each sampling date. The numbers of nymphs per leaf were recorded. Counts were repeated at 5- to 10-day intervals until the foliage began to dry with maturity.

Counts of plants were made to determine effects of the treatments on plant emergence. The counts were started when the first sprouts appeared above ground. The numbers of plants in 100 feet of row per replicate (400 feet of row per treatment) were recorded on each examination date.

RESULTS.—Aphid infestations did not develop in the plots at Davis and Stockton. At Shafter, in 1957, the green peach aphid was the only species that occurred in large numbers (table 1). At the first count, 63 days after planting, aphid populations were low, averaging 1.13 aphids per leaf in the checks. All of the materials reduced aphid numbers. However, it was obvious at this time that the demeton treatments were less effective than those with phorate or Di-Syston. Averages of 0.11 to 0.58 aphids per leaf were noted in the demeton plots, whereas in the phorate and Di-Syston plots, except for the seed treatment with 1.3 ounces of Di-Syston, averages ranged from 0.01 to 0.06 aphids per leaf. The demeton dip was somewhat more effective than the demeton-charcoal formulation. Aphid populations gradually increased in all of the plots as the season advanced but they increased more rapidly in the demeton plots. At 77 days after planting the counts were lower in the demeton plots than in the checks but it was obvious that little control was being obtained.

The treatments of seed pieces with charcoal formulations of phorate or Di-Syston began to lose effectiveness at the 77-day count although control was still satisfactory at this time.

The final aphid count was made 86 days after planting when the checks averaged 138.7 aphids per leaf. The treatment of seed pieces with phorate or Di-Syston were rapidly losing effectiveness but they were still less heavily infested than the checks. Plants from seed pieces treated with charcoal formulations of phorate at 1.3 and 2.6 ounces per 100 pounds of seed averaged 84.7 and 37.4 aphids per leaf, respectively, at this time. Those from seed pieces treated with Di-Syston at rates of 1.3 and 2.6 ounces of 50% charcoal formulation per 100 pounds of seed averaged 30.5 and 14.2 aphids per leaf, respectively.

The applications of phorate or Di-Syston granules provided outstanding control of the green peach aphid throughout this experiment. These treatments began to lose effectiveness after 86 days. At this time plots treated with the 2% Di-Syston granules averaged 2.83 aphids per leaf; and an average of 7.47 aphids per leaf occurred in plots treated with 2% phorate granules. There were thus approximately 49 times as many aphids in the checks as in the Di-Syston plots and almost 19 times more than in the phorate plots.

The tobacco flea beetle, *Epitrix hirtipennis* (Melsh.), was abundant early in the 1957 season at Davis. At 42 days after planting, the checks averaged 50.1 feeding scars per leaf. Control with demeton was not as good as with phorate or Di-Syston. The demeton emulsion dip treatment averaged 12.1 feeding scars per leaf. Averages of 22.6 and 30.0 scars per leaf were noted in the demeton charcoal seed treatments at rates of 2.6 and 1.3 ounces per hundred pounds of seed, respectively. Seed piece treatments with phorate and Di-Syston were more effective than the treatments with granules and with both types of application, phorate resulted in slightly better control than Di-Syston. Phorate seed piece treatments

Table 1.—Results with systemic insecticides applied to potato seed pieces or to the soil in preventing establishment of *Myzus persicae* on potato foliage. Shafter, California, 1957.

Treatment[a]		Average Number of Aphids per Leaf on Days Shown After Treatment[b]			
Material	Dosage	63	70	77	86
Check	—	1.13	7.47	24.79	138.71
Phorate 44%, on carbon	1.3 oz./100 lbs. seed	0.06	2.24	3.03	84.69
	2.6 oz./100 lbs. seed	0.02	0.31	1.86	37.40
Demeton 50%, on carbon	1.3 oz./100 lbs. seed	0.58	4.13	16.99	—
	2.6 oz./100 lbs. seed	0.32	2.27	11.13	—
Demeton 0.75%, E. Dip		0.11	1.73	9.79	—
Di-Syston 50%, on carbon	1.3 oz./100 lbs. seed	0.12	1.16	2.01	30.50
	2.6 oz./100 lbs. seed	0.01	0.17	0.65	14.15
Di-Syston (2%) granules	100 lbs./acre	0.02	0.02	0.22	2.83
Phorate (2%) granules	100 lbs./acre	0.02	0.05	0.37	7.47

[a] Seed cut and treated Feb. 4, 1957. Seed planted Feb. 5–6, 1957.
[b] Counts based on examination of 100 leaves per treatment. Only apterae and nymphs recorded.

Table 2.—Control of the tobacco flea beetle and *Empoasca filamenta* on foliage of potato plants by applications of systemic insecticides to seed pieces or to the soil. Davis, California, 1957.

Treatment		Average No. Flea Beetle Scars per Leaf 42 Days After Planting[a]	No. of Leafhopper Nymphs per 40 Leaves on Days Shown After Planting[b]			
Material	Dosage		77	87	94	101
Check	—	50.1	96	72	69	76
Phorate 44%, on carbon	1.3 oz./100 lbs. seed	2.7	15	57	65	94
	2.6 oz./100 lbs. seed	1.4	2	9	29	75
Demeton 50%, on carbon	1.3 oz./100 lbs. seed	30.0	68	73	90	91
	2.6 oz./100 lbs. seed	22.6	62	72	92	79
Demeton 0.75%, E. Dip		12.1	37	65	88	116
Di-Syston 50%, on carbon	1.3 oz./100 lbs. seed	3.9	7	35	47	58
	2.6 oz./100 lbs. seed	2.4	2	8	37	31
Di-Syston (2%) granules	100 lbs./acre	5.9	1	4	5	12
Phorate (2%) granules	100 lbs./acre	4.4	0	7	23	34

[a] Counts based on examination of 40 leaves per treatment.
[b] Ten leaves examined per replicate.

(44% on charcoal, 1.3 and 2.6 ounces per 100 pounds of seed) resulted in averages of 2.7 and 1.4 scars per leaf, respectively, whereas the Di-Syston seed piece treatments (50% on charcoal, 1.3 and 2.6 ounces per 100 pounds of seed) resulted in respective averages of 3.9 and 2.4 scars per leaf. An average of 4.4 scars per leaf occurred with the 2% phorate granules applied to the soil while the 2% Di-Syston granules averaged 5.9 scars per leaf.

The leafhopper, *Empoasca filamenta* DeL., occurred in the plots at Davis in 1957 and 1958 (tables 2 and 3). The heaviest infestations were encountered in 1958. In the 1957 experiments, demeton failed to control this leafhopper. Seed piece treatments with phorate or Di-Syston in both experiments were not as effective as applications of granules. With the seed piece treatments in 1957, dosages of 2.6 ounces of the carbon formulations per 100 pounds of seed were more effective than 1.3 ounces. These treatments with carbon formulations were effective for about 87 days after planting. In 1958, the carbon formulations applied at 2 ounces per 100 pounds of seed were not highly effective 73 days after planting. Applications of granules of phorate or Di-Syston gave about the same excellent leafhopper control for 97 to 100 days after planting.

INSECTICIDE RESIDUES ON TUBERS RESULTING FROM TREATMENTS.—Samples of tubers from the phorate treatments in 1958 were taken 74 days after planting at Davis and 91 days after planting at Stockton. These were analyzed for phorate by the Pesticide Residue Research Laboratory at Davis using a cholinesterase inhibition method. The method was sensitive to 0.005 p.p.m. No detectable residues were found in any of the samples submitted.

Table 3.—Control of *Empoasca filamenta* on potatoes with systemic insecticides applied to the cut seed and to the soil at planting. Davis, California, 1958.

Treatment[a]		Nymphs per 40 Leaves[b] Days After Planting			
Material	Dosage	73	78	87	97
Check	—	333	362	255	200
Phorate 44%, on carbon	2 oz./100 lbs. seed	228	347	335	—
Di-Syston 50%, on carbon	2 oz./100 lbs. seed	159	164	116	—
Di-Syston 2.5%, granules	80 lbs./acre	25	21	12	11
Phorate 2.0%, granules	100 lbs./acre	2	2	3	10

[a] Seed cut April 16, treated April 17, planted April 18, 1958.
[b] Ten leaves examined per replicate on each sampling date.

EFFECTS OF TREATMENTS ON PLANT GROWTH.—During the course of these experiments it was observed that certain of the treatments affected emergence and growth of plants and the results recorded are shown in tables 4 and 5. In the 1957 experiments at Shafter and Davis, treating the seed pieces with demeton both as powders and as a dip appeared to stimulate plant growth. As noted in counts made in both trials (table 4), plants from demeton-treated seed pieces emerged ahead of all other treatments including the untreated checks. On the other hand, charcoal formulations of phorate or Di-Syston, especially at the higher dosages of 2.6 ounces per 100 pounds of seed, greatly retarded plant emergence. At the conclusion of plant counts, 56 days after planting at Shafter and 44 days after planting at Davis, plants grown from seed pieces treated with phorate or Di-Syston were smaller in size and it was evident that minor reductions in stand had occurred with all treatments when compared with the untreated checks. In the experiments at Davis and Stockton in 1958, the treating of seed pieces with charcoal formulations of phorate or Di-Syston resulted in serious injury. As shown in table 5, reductions in numbers of plants of 19.2% and 11.0% occurred with phorate and Di-Syston, respectively, in the Davis experiment. At Stockton where the potatoes were grown in peat land, frequent difficulties are encountered with seed piece decay. In this experiment only 283 plants developed in 400 feet of row in the untreated checks. Where charcoal formulations of phorate and Di-Syston were applied directly to the seed pieces, reductions in plant numbers of 44.9% and 59.0%, respectively, occurred.

In the 1957 trials there was relatively little difference in final plant counts between applications of granules and seed piece treatments. Although the granules appeared to have less effect on delaying plant emergence there were slight reductions in stand with these treatments. In the 1958 tests, applications of granules appeared to have little or no adverse effects on emergence and plant growth. The placing of the insecticides in bands away from the seed pieces undoubtedly accounted for this.

Some cupping and marginal burn of basal leaves were observed soon after plant emergence in the phorate and

Table 4.—Effect on plant emergence resulting from applications of systemic insecticides to potato seed pieces or to the soil in field trials at Shafter and Davis, California, 1957.

Treatment		No. of Plants Emerged per 400 Feet of Row on Days Shown After Planting[a]							
		Shafter[b]				Davis[c]			
Material	Dosage	37	42	56	Reduction (%)	27	32	44	Reduction (%)
Check	—	177	353	384	—	153	304	379	—
Phorate 44% C	1.3 oz./100 lbs. seed	129	335	375	2.34	55	232	350	7.65
	2.6 oz./100 lbs. seed	24	164	342	10.94	28	170	340	10.29
Di-Syston 50%, C	1.3 oz./100 lbs. seed	129	347	377	1.82	28	203	347	8.44
	2.6 oz./100 lbs. seed	44	220	357	7.03	5	99	345	8.97
Demeton 50%, C	1.3 oz./100 lbs. seed	300	362	378	1.56	247	355	376	0.79
	2.6 oz./100 lbs. seed	258	343	361	5.99	257	362	378	0.26
Demeton 0.75, E.C.	0.75% Emul. Dip	213	310	336	12.50	229	328	367	3.17
Di-Syston (2%) granules	100 lbs./acre	137	306	361	5.99	100	255	360	5.01
Phorate (2%) granules	100 lbs./acre	102	271	348	9.36	77	212	355	6.33

[a] Counts based on examination of 100 feet of row in each of four plots. Seed pieces spaced 1 foot apart in row.
[b] Seed cut and treated Feb. 4; seed planted Feb. 5–6, 1957.
[c] Seed cut March 27, treated March 28, planted March 29, 1957.

the Di-Syston seed piece treatments. This was not serious and did not progress as the plants increased in size.

DISCUSSION.—Perhaps the greatest potential use of treatments involving systemic insecticides is in the production of certified seed potatoes. In order to produce seed potatoes the grower must meet certain certification requirements with respect to the presence of viruses and other diseases. Since insects, especially aphids, are vectors of most of the potato viruses, insecticides that will control these vectors are of importance. Perhaps the most important consideration in evaluating an insecticide under these circumstances is whether the vectors are killed before transmitting the viruses, a phase not included in the scope of the experiments reported in this paper. Hille Ris Lambers, *et al.* (1953) and Hoyman (1958) have shown that systemic insecticides are of little value in controlling nonpersistent potato virus-Y. With respect to potato leafroll, a persistent type virus, Hille Ris Lambers *et al.* (1953) found that periodic spraying of potato plots with demeton greatly reduced the spread of leafroll within the field but did not prevent leafroll infection which was brought into the plots by aphids that obtained the virus elsewhere. Klostermeyer (1953), using sprays of demeton and schradan, held aphid populations to a very low level, but found that these materials resulted in more leafroll spread per unit of aphid population than the more rapidly acting contact insecticide, parathion.

It is necessary for the aphids to feed in order to obtain a lethal dose of insecticide; and while feeding, the aphids if infective can transmit the virus. It seems therefore that there is little that can be accomplished with systemic insecticides to prevent infection by disease-bearing aphids that may be moving into a field. However, in the case of leafroll, if aphids can be prevented from reproducing on and moving from diseased plants within the field to healthy ones, the diseased plants will present little hazard to others in the field. It appears from the results obtained with soil applications of phorate and Di-Syston that it may be possible to minimize the importance of leafroll-infected potato plants and thus materially reduce the spread of this virus within the field.

In California the green peach aphid is generally abundant in the early spring. Populations decline rapidly with the onset of warm weather in the interior valleys and they do not reappear until the return of cool weather in the fall. The outlook for controlling aphids for a season with

Table 5.—Plant emergence resulting from applications of phorate or Di-Syston to seed potatoes or to the soil in field trials at Davis and Stockton, California, 1958.

Treatment		No. of Plants Emerged per 400 Feet of Row on Days Shown After Planting[a]							
		Davis[b]				Stockton[c]			
Material	Dosage	21	27	36	Reduction (%)	19	25	28	Reduction (%)
Check	—	255	351	354	—	170	267	283	—
Phorate 44%, C	2.0 oz./100 lbs. seed	78	259	286	19.21	63	143	156	44.88
Di-Syston 50%, C	2.0 oz./100 lbs. seed	120	296	315	11.02	42	106	116	59.01
Phorate (2%) granules	100 lbs./acre	275	352	365	0.00	159	226	238	15.90
Di-Syston (2.5%) granules	80 lbs./acre	283	353	354	0.00	181	263	263	7.07
Phorate (2%) granules	200 lbs./acre	—	—	—	—	170	249	262	7.42
Di-Syston (2.5%) granules	160 lbs./acre	—	—	—	—	189	248	258	8.83

[a] Counts based on examination of 100 feet of row in each of four plots. Seed pieces spaced 1 foot apart in row.
[b] Seed cut and treated April 17, planted April 18, 1958.
[c] Seed cut and treated May 7, planted May 8, 1958.

a single application of phorate or Di-Syston at planting time is promising on spring crops. It is possible to protect the plants early in the season for approximately 80 days after which time the aphid populations normally decline with rising summer temperatures. Where a late or fall crop is being grown, a single application at planting probably would not provide control for the last 30 days of growth. Whether a side dressing applied 30 to 40 days after planting would extend protection over this period has not been determined.

Phorate or Di-Syston applied to the soil with fertilizer or seed at the time of planting considerably reduces populations of the green peach aphid, leafhoppers and the tobacco flea beetle on potato for periods of 80 to 85 days after planting. There appears to be little difference in the effectiveness of the two insecticides in controlling these pests. Treating cut seed pieces with these insecticides is hazardous from the standpoint of plant injury, especially under adverse growing conditions. The most effective and safe method of using the materials on potatoes is in the form of granules applied with the fertilizer. Further studies on combining the insecticides with the fertilizer granules thereby eliminating the need for a separate carrier for the insecticide is desirable. Two pounds of actual toxicant per acre applied as granules appear to be a minimum dosage for control of aphids and leafhoppers. Increased dosages and improvements in placement of the insecticide may provide even better control.

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Studies with Several Systemic Insecticides for the Control of Azalea Lace Bugs

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ABSTRACT

Dimethoate, Di-Syston® (*O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorodithioate) and phorate granules applied to the soil surface under azalea plants controlled two broods of azalea lace bugs (*Stephanitis pyrioides* Scott) with a single application. The initial control brought about by the use of these chemicals seems to be caused by vapors. Phorate did not give systemic control when worked into azalea beds.

No phytotoxicity was observed from any treatment nor was there any deterrent to the rooting response of cuttings treated with demeton, Di-Syston or phorate.

The azalea lace bug, *Stephanitis pyrioides* Scott, is considered the most common and most important insect pest of azaleas in Maryland. The insecticides currently recommended are effective if properly used, but several spray applications are needed during the growing season. Tests with systemic insecticides were carried out with the idea that the number of treatments might be reduced and control simplified.

Previous studies at the University of Maryland (unpublished) showed that phorate, schradan, dimefox and demeton as drenches, killed lace bugs on greenhouse-grown azaleas. It has been shown by Hopkins *et al.* (1959) that long periods of aphid control on cotton have been obtained with treatments of granulated phorate. Schread (1959) obtained good control of both azalea and andromeda lace bugs in field tests using phorate granules. Cook's (1959) experiments, with granulated phorate on alfalfa, to control the pea aphid, indicated that the aphid mortality appeared to be entirely from fumigation.

This paper reports the results of several field experiments conducted over a period of 2 years.

1958 TESTS.—The 1958 prefatory investigations were concerned with the effects of Di-Syston® (*O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorodithioate) and phorate, utilized as granules and emulsifiable sprays, and demeton only as an emulsifiable spray. The tests were made in a commercial nursery, using 3-year-old azaleas of the Hinodegiri variety. The plants were grown in three beds with approximately one plant per square foot. A 40-square-foot section of each bed was set aside as an untreated control plot separated from the treated plot by one row of azaleas. Sprays were applied with a compressed-air sprayer and directed to the upper surface of the leaf. One tablespoonful of the emulsifiable insecticide was mixed with 2 gallons of water and applied to 75 square feet of the designated plot. The granulated insecticides were broadcast by hand at the rate of 1 pound per 50 square feet of bed.

The pretreatment counts (table 1) represent the total

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Table 1.—Experimental control of azalea lace bugs treated August 5, 1958.

Insecticide and Concentration (%)	Insecticidal Activity: No. of Lace Bugs After Days Indicated[a]			
	Pretreatment	2	35	49
Sprays				
Demeton, 0.05	53	0	3	6
Di-Syston, 0.05	156	0	0	4
Phorate, 0.1	72	0	6	8
Granules				
Di-Syston, 2 (.016 oz.)[b]	48	0	0	0
Phorate, 8 (.025 oz.)[b]	52	0	0	0
Untreated	75	0	2	30

[a] Pretreatment counts on three plants; posttreatment counts on eight plants except untreated; untreated represents total lace bugs on 40 plants.
[b] Ounces of active ingredient per plant.

number of lace bugs (both adults and nymphs) found on three randomly selected plants from each plot. The posttreatment counts represent the total number of lace bugs found upon examination of eight randomly selected plants per plot except in the check plot where all (approximately 40) plants were examined. The number of plants examined after treatment was increased to show the extent of control, particularly in the check plots. Demeton, Di-Syston and phorate as sprays gave excellent control of lace bugs. The Di-Syston and phorate granules completely controlled lace bugs for more than 60 days, giving a longer period of control than the sprays. The check plots were down wind from the granulated insecticide treatments, and the control observed here was believed to be the result of toxic vapors. The odor of Di-Syston and phorate granules persisted for more than 4 weeks and could be minutely detected 7 weeks after treatment. The granules had not completely disappeared when the plants were dug the following March.

With the assistance of the University of Maryland Department of Horticulture, 100 cuttings were taken 7 days after treatment from each of the plots to determine if there was any effect on the rooting response of cuttings. These cuttings were examined in October after being in the greenhouse under intermittent mist for 10 weeks. The results showed no observable difference between cuttings taken from the control and treated plots.

1959 TESTS.—*Phorate.*—Many people consider the odor of phorate strongly objectionable, especially when applied near dwellings. An experiment was devised to subdue this odor, by incorporating the insecticides into the soil, containing a large amount of peat moss, and to make further observations concerning the role of phorate vapors in lace bug control.

An area covering one-half acre was worked into azalea beds, and the area was separated into four sections. One section was designated as an untreated check plot, and the other sections received single treatments of either 4, 8, or 9 pounds of actual phorate per acre. Two per cent phorate granules were applied with a fertilizer spreader. A rototiller was used to work the phorate into the top 5 or 6 inches of soil. The day after the soil treatment, two-year-old cuttings were transplanted into the beds. On this date (May 29) damage was observed on the rooted cuttings planted in the check plot, but no lace bugs were found. Some of the varieties planted in the treated plots were heavily infested with lace bug adults and nymphs. Fifteen days after treatment, spot checks were made indicating a light infestation in the untreated plot. Some sections of the treated plots were heavily infested with lace bugs and corresponded to the sections where heavy infestations were found in the cutting transplants. Counts made in part of a plot containing Litany and Purple Splendor varieties averaged 40 lace bugs and nymphs per plant. This plot had been treated at the rate of 8 pounds of phorate per acre. At this point in the test no control could be observed.

Before a systematic collection of data could be taken from the test area the nurseryman was forced to dust the entire nursery, using malathion, because of a serious local outbreak of Japanese beetles. The malathion treatment introduced a variable which could not be overcome; therefore, no further data were taken.

Observations were continued, however, throughout the growing season. Phorate gave no observable phytotoxicity, nor was there any observable improvement in the growth of the plants. A heavy infestation of the two-spotted spider mite (*Tetranychus telarius* (L.)) was found 15 weeks after treatment in the 9-pound per acre treatment indicating ineffective systemic control at that time.

In the data available from the phorate tests of 1958 and 1959 it seems unlikely that the control obtained with broadcasted granules was from systemic action, since complete control was observed within 2 days. The lack of control in plots where phorate was worked into the soil lends some evidence to the fumigation theory. The lack of systemic control in 1959 might be laid to these factors: too little phorate in the root area or bound by organic material; more than 15 days required by azaleas to pick up the phorate in amounts great enough to control lace bugs.

Dimethoate.—In May 1959, a heavy infestation of lace bugs, averaging 7 adults and nymphs per leaf, was observed in a landscape planting of azaleas. Eight per cent granulated dimethoate was broadcast by hand to these plants on the basis of 1 pound of the 8% granules per 100 sq. ft. One week later no live lace bugs could be found. Two plants, one at each end of the planting, were untreated, and no lace bugs were observed on these plants. These plants were observed periodically throughout the summer, and no lace bugs were found until September 21 when the untreated check plants were found to have a very light infestation of adult lace bugs. An occasional adult was found on the treated plants. By October 5, lace bugs were numerous. Using the criterion of Schread (counting all the lace bugs that could be observed in 5 minutes) there were five lace bugs on the treated plot compared with 49 on the untreated. These counts did not depict the picture as was observed from the extensive damage present in the treated plot. This treatment appears to control the first and second broods of lace bugs. The results with this insecticide parallel those of Di-Syston and phorate when applied as granules to the soil surface.

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Comparative Acaricidal Activity of Tedion, Ovex, and Chlorbenside Against Two Strains of the Two-Spotted Spider Mite[1]

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ABSTRACT

Tedion (2,4,4′,5-tetrachlorodiphenyl sulfone), ovex, and chlorbenside were tested in the laboratory against two strains of two-spotted spider mite (*Tetranychus telarius* (L.)). The fecundity of neither strain was affected by their contact or residual action on the adults. Tedion residues were highly toxic to eggs and larvae of both strains when adult mites fed and oviposited on treated leaves. Ovex and chlorbenside residues were only slightly effective as ovicides. Both had a high larvicidal activity against the susceptible strain, but were less effective against the resistant strain. Tedion-treated females produced eggs that were apparently healthy, but most of the eggs died. Most of the eggs and larvae from ovex- or chlorbenside-treated mites were not affected.

In recent years several investigators have reported Tedion (2,4,4′,5-tetrachlorodiphenyl sulfone), ovex, and chlorbenside specific in toxic action to eggs and larvae of spider mites.

Cranham *et al.* (1953) found chlorbenside toxic to eggs and young nymphs of the two-spotted spider mite (*Tetranychus telarius* (L.)) but not to adults. Persistent residual properties against immature stages reduced active infestations to low levels in 2 to 3 weeks. In laboratory tests Armstrong *et al.* (1955) found ovex and chlorbenside highly effective against the eggs of tetranychid mites. Other workers reported Tedion to be effective against the eggs, larvae, and nymphs. The eggs in the ovaries of adult females were also affected (Huisman *et al.* 1955, Meltzer 1956, Ferguson 1958).

This paper reports on laboratory experiments conducted at Beltsville, Md., during 1958 in which these acaricides were compared on two strains of the two-spotted spider mite as to (a) their effect on eggs and larvae produced by treated females, (b) the effect of residues on eggs produced by untreated females, and (c) their effect on fecundity.

GENERAL PROCEDURE.—The test mites had different LD-50's of malathion (Taylor & Smith 1956). One strain, known as the Massachusetts colony, was collected from carnations near Waltham in October 1951, and the second strain, the Cranbury colony, was obtained from roses in New Jersey in February 1949. The Massachusetts colony is susceptible to organic-phosphorus compounds, whereas the Cranbury-1 colony is resistant. All the stock colonies had been maintained on lima bean plants without exposure to acaricides.

Lima bean plants were trimmed of all but the two primary leaves and set individually in 150-ml. bottles containing the nutrient solution of Hyponex. The petiole of each leaf was banded with lanolin to keep the mites from wandering off the plant, and the neck of each bottle was smeared with a sticky barrier (Deadline) as insurance against possible contamination with mites from other sources. The mites were transferred from the stock colony to test plants with a fine sable-hair brush.

The plants were treated by dipping in one of the acaricides made up at the following rates of wettable powder per 100 gallons of water: 2 pounds of 25% Tedion, 1 pound of 50% ovex, and 2 pounds of 20% chlorbenside. All experiments were conducted in a randomized block design with four replications.

EFFECT ON EGGS AND LARVAE PRODUCED BY TREATED MITES.—In experiments to determine the effect on eggs and larvae produced by treated mites, groups of 20 females of each strain were transferred to clean plants, where they were treated with one of the three acaricides. In one experiment they were allowed to feed and reproduce for 1 day and then transferred successively to clean plants 1, 3, and 4 days after treatment. The females were exposed to the acaricide by wetting plus 24 hours on the dried residue. In another experiment the mites were removed from the treated plants as soon as they were dry and transferred to clean plants at the end of 2, 6, 8, and 9 days. The living and dead eggs and immature mites were counted 7 days after females were removed.

The mortalities of eggs and larvae produced by treated females are shown in table 1. In the first experiment high egg mortality resulted from the Tedion treatment. Eggs deposited by both strains were nonviable when the females were allowed to oviposit on the plants just prior to dipping and were held on the same plants for 1 day. All these eggs were exposed to the Tedion residues for 7 days. A high percentage of those produced by Tedion-treated females after 1 to 3 and 3 to 4 days also failed to hatch. High egg mortality also resulted from the ovex treatment (0 to 1). In most cases fully developed larvae were seen dead but within the chorion. However, eggs from the same females were less affected when deposited on untreated foliage 1 day or longer after treatment.

In the second experiment the eggs were not exposed to contact action or to residues of the acaricides. A high percentage of eggs deposited by Tedion-treated females during the first 6 days were non-viable, and the effect on the viability of these eggs was evident for 9 days. The

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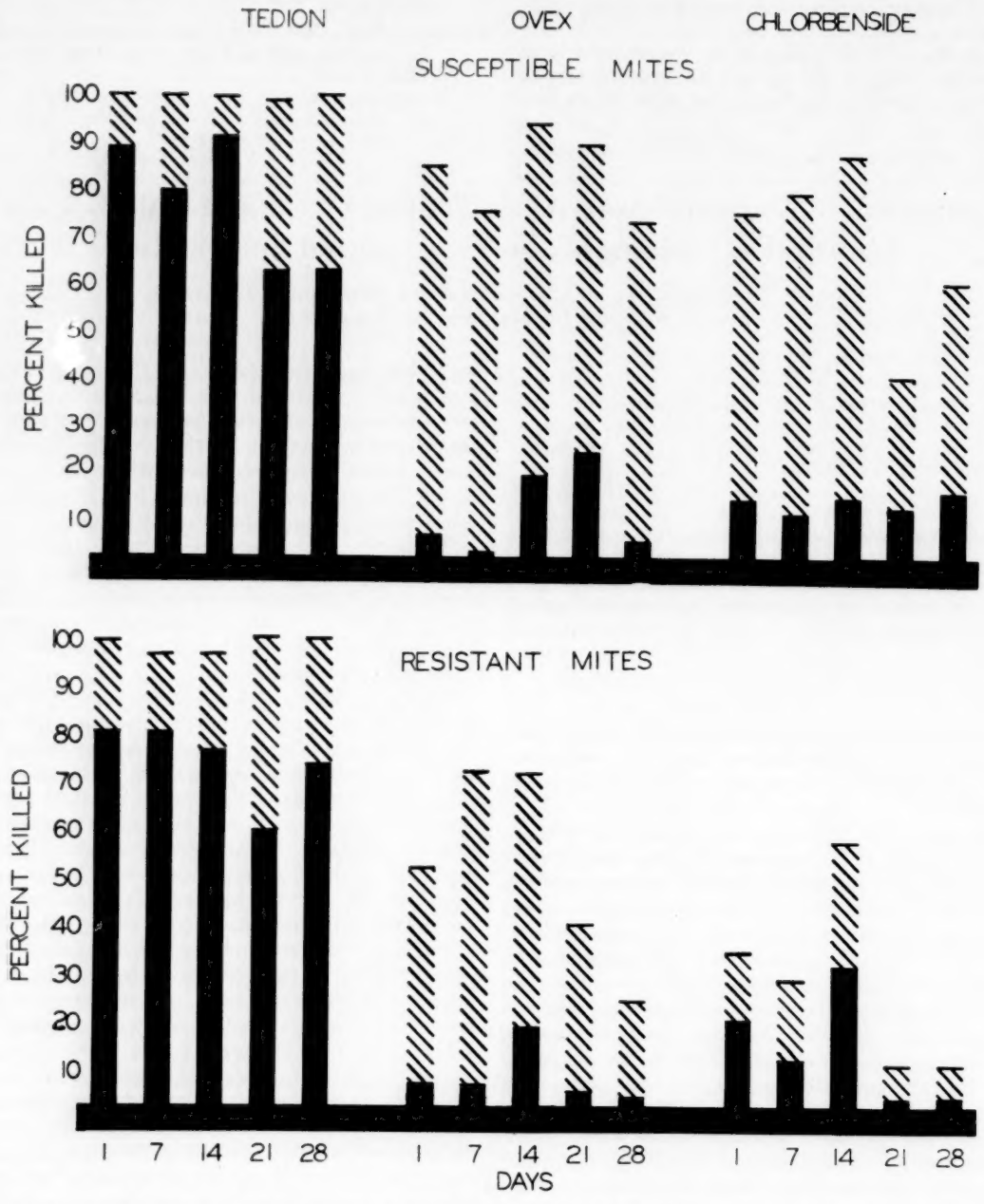

FIG. 1.—Acaricidal activity of Tedion, ovex, and chlorbenside residues of different ages in two strains of the two-spotted spider mite. Solid black bars represent dead eggs and cross-hatched bars dead larvae.

high mortality of eggs produced by Tedion-treated mites moved to clean plants supports the finding that the egg is affected in the ovary (Meltzer 1956). This type of activity by Tedion is not so clearly indicated when the mites feed and oviposit upon treated plants, since the effect on the eggs cannot be considered independent of the effect on the adults.

Eggs deposited on clean foliage by ovex-treated females up to 8 days after treatment were less affected. Chlorbenside was similar to ovex against eggs of both strains. The larvicidal action of all three materials was low after the first transfer.

EFFECT OF RESIDUES ON EGGS DEPOSITED BY UNTREATED FEMALES.—The effect of residues on eggs deposited by untreated females was determined by confining mites for 24 hours on treated plants with residues of different ages (table 2). A high percentage of the eggs deposited on Tedion residues failed to hatch. The effectiveness against resistant mites declined somewhat after 7 days. Most eggs deposited by untreated females on ovex residues were viable. On 1-day-old residues many of the dead larvae were seen in the cracked chorion, but most of the larvae emerged from the eggs on the older residues. Ovex retained its larvicidal effectiveness against

Table 1.—Per cent mortality of eggs and larvae produced by two strains of two-spotted spider mites treated with different acaricides.

Days After Treatment	Susceptible Mites		Resistant Mites	
	Eggs	Larvae	Eggs	Larvae
Experiment 1				
Tedion				
0–1	88	12	91	9
1–3	97	1	86	4
3–4	73	1	86	1
Ovex				
0–1	99	0	95	1
1–3	13	3	24	4
3–4	8	1	9	2
Experiment 2				
Tedion				
0–2	85	6	91	2
2–6	73	1	61	1
6–8	33	1	22	3
8–9	27	1	27	1
Ovex				
0–2	8	29	16	10
2–6	5	11	1	4
6–8	1	2	2	2
8–9	10	2	7	2
Chlorbenside				
0–2	4	11	24	13
2–6	4	9	9	3
6–8	1	1	2	1
8–9	9	1	11	1

both strains for 7 days, but after 14 days it was less effective and after 21 days was markedly reduced.

A similar experiment was conducted with untreated mites transferred to plants with ovex, Tedion, or chlorbenside residues of different ages, and the results are shown in figure 1. Eggs of both strains deposited on Tedion residues were affected for 28 days. Egg mortality was reduced after 14 days but most of the hatching larvae were killed. Most of the eggs deposited on ovex residues by both strains were viable. More than two-thirds of the hatching larvae of the susceptible strain were killed up to 28 days, but ovex was less effective against the resistant strain. Most of the eggs also hatched on the chlorbenside residues. Although a high larval mortality of the susceptible strain was evident for 14 days, chlorbenside was inferior to ovex against both strains.

Table 2.—Per cent mortality of eggs and larvae produced by two strains of two-spotted spider mites exposed to Tedion and ovex residues of different ages.

Age of Residue (days)	Susceptible Mites		Resistant Mites	
	Eggs	Larvae	Eggs	Larvae
Tedion				
1	84	9	86	11
7	89	11	88	11
14	83	8	78	8
21	93	1	76	2
Ovex				
1	7	84	4	90
7	9	90	4	87
14	5	73	3	67
21	5	41	3	39
L.S.D. (19:1)	6	12	6	12

Table 3.—Number of eggs per week per female of two strains of two-spotted spider mites fed on lima bean plants treated with different acaricides.

Acaricide	Susceptible Mites	Resistant Mites
Tedion	47	36
Ovex	47	36
Chlorbenside	47	37
Untreated	50	38

EFFECT ON FECUNDITY.—The effect of acaricides on the rate of egg production was determined after treating 20 females of each strain with Tedion, ovex, or chlorbenside and moving them individually to treated lima bean plants. The number of progeny obtained after 1 week indicate no difference between the number of eggs deposited on treated and untreated plants (table 3). Mites of the susceptible strain deposited more eggs than those of the resistant strain. Similar results were obtained in an earlier study by Lehr & Smith (1957).

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Factors Affecting the Tolerance of Boll Weevils to Calcium Arsenates[1]

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ABSTRACT

Boll weevils (*Anthonomus grandis* Boh.) emerged from cotton bolls were more tolerant to tricalcium arsenate than those emerged from squares. Their wet weight and fat content were also greater. When field-collected weevils were exposed to calcium arsenate in petri dishes with or without cotton squares for food, the insects that died first had lower body weight and fat content than those that died later. Tricalcium arsenate and commercial calcium arsenate were found to kill weevils when ingestion of the poisons was made impossible, but their effectiveness was reduced by one-half. Weevils treated with calcium arsenates lost weight more rapidly under desiccation than untreated live or dead weevils. The mortality of treated insects decreased as the humidity increased, but that of untreated insects showed little difference. These results indicate that under field conditions, when the evaporation rate is higher than that in the laboratory, the desiccating action of calcium arsenates may be responsible for partial control of the insects.

Calcium arsenate was the best arsenic compound tested before 1920 for control of the boll weevil (*Anthonomus grandis* Boh.) (Coad & Cassidy 1920) and was the principal insecticide used for this purpose until the advent of chlorinated hydrocarbon insecticides, such as BHC and toxaphene, which were first demonstrated to be effective against this insect by Ivy *et al.* (1947). Aldrin, dieldrin, and endrin were recommended for boll weevil control at the Conference of State and Federal workers (1953), and the organic phosphorus insecticides, methyl parathion, Guthion® (*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3-(4*H*)-ylmethyl) phosphorodithioate), and malathion, were recommended at the Conference 3 years later (1956). The occurrence of boll weevil resistance to the chlorinated hydrocarbons in several localities of the Cotton Belt (Roussel & Clower 1955, 1957; Walker *et al.* 1956; Fye *et al.* 1957) renewed interest in the use of calcium arsenate.

Weevils reared from cotton squares were found to be more susceptible to toxaphene than those reared from bolls (Gaines & Mistric 1952). Reiser *et al.* (1953) found that weevils became more tolerant to toxaphene and to calcium arsenate as their body weight and fat content increased with the advancement of the growing season. Brazzel *et al.* (1957) reported that adult and larval foods affected the fat content of adults. Experiments were conducted at Brownsville, Texas, to determine whether weevils reared from squares and bolls differ in their tolerance to calcium arsenate and whether this tolerance is correlated with body weight and fat content.

Calcium arsenate is considered a stomach poison. However, preliminary results showed that adult weevils with sealed mouth parts were killed by the insecticide, and that they became brittle immediately following death. These observations indicated that calcium arsenate caused the insects to dehydrate rapidly and die without ingesting the poison. Further experiments were therefore conducted to determine the presence of desiccating action and the effects of humidity on this action.

GENERAL MATERIALS AND METHODS.—Commercial calcium arsenate, and monocalcium, dicalcium, and tricalcium arsenates[3] were used.

Field-collected weevils and weevils emerged from infested squares or bolls in the laboratory were tested separately. Newly emerged weevils were fed on cotton bolls or squares for 2 to 3 days before they were tested.

Individual potted cotton plants or two to four petri dishes (3.5 or 5 inches in diameter) were dusted with weighed amounts of the insecticide in a dust chamber. The dust chamber consisted of a glass cylinder, 12 inches in diameter and 26 inches tall, mounted on top of a metal drum 14 inches in diameter and 24 inches tall. The drum was open at the top and equipped with a side door. The glass cylinder had a metal cover with a hole in the center through which the dust was dispersed by means of an air blast. Both the metal cover and the drum were grounded to avoid the effect of static electricity on the settling of dust particles. The dust was allowed to settle for 2 minutes before the plants or petri dishes were removed.

The test insects were either confined in cages 8×9×12 inches, each containing one dusted plant, or in dusted petri dishes. The number of dead insects was recorded after 1, 2 or 3 days. Untreated insects were used as checks. Slopes of the dosage-mortality curves and median lethal dosages (LD-50's) were determined by Finney's method (1952).

Dry weights of newly emerged weevils and 2-day-old weevils that had fed on cotton bolls were determined after drying in an oven at 70° C. to constant weight. Fat contents were determined by extracting the dried, coarsely ground insects with ether for 6 hours in a Soxhlet apparatus, following the method of Brazzel *et al.* (1957).

All experiments were conducted in the laboratory where the temperature was 80 ± 2° F. and the relative humidity varied from 50% to 70%, except as indicated in experiments comparing various constant humidities.

TOLERANCE OF BOLL- AND SQUARE-REARED WEEVILS.—Weevils emerged from bolls and squares were tested separately in order to determine their tolerance to tricalcium arsenate. Potted plants were dusted at four dosage levels. Thirty 2-day-old weevils were confined in each cage with a dusted plant. Sixty each of the boll- and square-reared weevils were used for each dosage. Mortality data given in table 1 indicate that at the end of 2- and 3-day test periods, the boll-reared weevils were 2.7 and 6.3 times, respectively, as tolerant as the square-reared ones.

Body weight and fat content of newly emerged weevils and 2-day-old weevils emerged from squares and bolls were determined in order to correlate these two factors

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[2] In cooperation with the Texas Agricultural Experiment Station.
[3] Monocalcium, dicalcium, and tricalcium arsenates were furnished by the American Smelting & Refining Company, South Plainfield, N. J.

Table 1.—Mortality of boll- and square-reared boll weevils after exposure to cotton plants dusted with tricalcium arsenate.

Exposure (days)	Net Per Cent Mortality at Indicated Dosage (mg.)				LD-50	Slope
	40	80	160	320		
Boll-reared weevils						
2	9.2	21.1	34.5	58.4	249	1.68
3	21.1	39.8	47.5	66.7	152	1.30
Square-reared weevils						
2	21.4	57.1	65.5	78.0	93	1.63
3	63.1	78.6	86.9	95.2	24	1.44

with the tolerance of the insects to the insecticide. The results, given in table 2, indicate that the boll-reared weevils were heavier and had more fat than the susceptible, square-reared weevils, and that 2-day-old weevils had higher body weight and lower fat content than those newly emerged. However, Brazzel *et al.* (1957) observed continuous increase in fat content of boll weevils during the first few days of feeding.

In other experiments weevils were confined in 5-inch-diameter petri dishes that had been dusted with calcium arsenate. Weevils collected from fields at Brownsville, Texas, and near Tampico, Tamps., Mexico, were used in experiments 1 and 2, respectively. Each dish was dusted with 340 mg. of the insecticide for experiment 1 and 140 mg. for experiment 2. Ten treated dishes containing 100 weevils per dish were used in each experiment. Squares were furnished as food in experiment 1 only. Dead insects were removed from the dishes periodically, pooled for each experiment, and their body weight and fat content determined. The results, shown in table 3, indicate that the insects that died first had lower body weight and fat content than those that died later. Weevils that survived the tests were the heaviest and had the highest fat content.

CONTACT ACTION.—Boll-reared weevils were used to determine whether calcium arsenates were toxic as contact poisons when ingestion of the material was made impossible. In two experiments weevils were treated in the following manner before exposure to the insecticide: The portion of the snout from the tip to a point just beyond the base of the antenna was dipped in plastic cement; after drying, the cement formed a hard sheath which the weevil could not remove.

In the first experiment, 20 each of treated and untreated weevils were confined in the same cage containing a cotton plant dusted with tricalcium arsenate. Each dosage was replicated twice. Average mortalities of the weevils in 24 and 48 hours are given in table 4. In the second experiment, 20 treated or untreated weevils were confined in a petri dish dusted with one of the insecticides. Mortalities after 18-hour exposure to monocalcium, dicalcium, tricalcium, and commercial calcium arsenates are given in table 5.

The LD-50 values for weevils with sealed mouth parts, exposed to calcium and tricalcium arsenates, were about twice as high as those for weevils with normal mouth parts. In other words, the absence of insecticidal action

Table 2.—Body weight and fat and water contents of boll weevils that emerged from cotton bolls and squares.

Insect	No. of Insects	Average Body Weight (mg.)		Per Cent Water	Per Cent Fat	
		Wet	Dry		Wet Basis	Dry Basis
Emerged from squares:						
Newly emerged	299	8.64	2.12	75.5	1.74	7.04
2-day-old	300	11.69	2.82	76.0	1.37	5.72
Emerged from bolls:						
Newly emerged	199	14.38	4.46	69.0	3.33	10.68
2-day-old	205	15.11	4.42	70.8	2.36	8.08

Table 3.—Mortality, body weight, and fat content of field-collected boll weevils exposed to calcium arsenate in petri dishes.

Condition of Insect in or After Hours Shown	No. of Insects	Accumulated Mortality (%)	Average Dry Body Weight (mg.)	Fat (%)
Experiment 1 (320 mg.)				
Dead in 16	351	35.1	5.29	12.9
Dead in 24	171	52.2	5.68	12.5
Dead in 41	352	87.4	6.25	18.5
Alive after 41	125	—	6.74	21.2
Experiment 2 (140 mg.)				
Dead in 24	278	28.0	4.44	4.88
Dead in 48	343	62.6	5.57	6.41
Dead in 72	260	88.8	6.25	8.21
Alive after 72	111	—	6.98	10.82

Table 4.—Mortalities of boll weevils with normal or sealed mouth parts when exposed to cotton plants dusted with tricalcium arsenate.

Exposure (hours)	Net Per Cent Mortality at Indicated Dosage (mg.)				LD-50	Slope
	40	80	160	320		
Normal mouth parts						
24	11.1	16.7	44.5	47.2	301	1.43
48	17.6	50.0	73.5	91.2	84	2.43
Sealed mouth parts						
24	0	10.5	34.2	31.6	570	1.29
48	0	8.8	70.5	61.8	187	2.74

Table 5.—Mortalities of boll weevils with normal or sealed mouth parts after exposure in petri dishes for 18 hours to calcium arsenates.

Arsenate	Per Cent Mortality at Indicated Dosage (mg.)				LD-50	Slope
	15	30	60	120		
Normal mouth parts						
Tricalcium	5.3	7.9	71.1	92.1	50	3.68
Calcium	2.5	2.5	25.0	75.0	83	4.14
Dicalcium	7.9	0	18.4	23.7	938	.78
Monocalcium	23.7	26.3	24.2	44.7	215	.67
Sealed mouth parts						
Tricalcium	0	5.3	42.1	73.7	77	3.73
Calcium	0	0	7.5	32.5	165[a]	3.21[a]

[a] Estimated from a curve fitted visually.

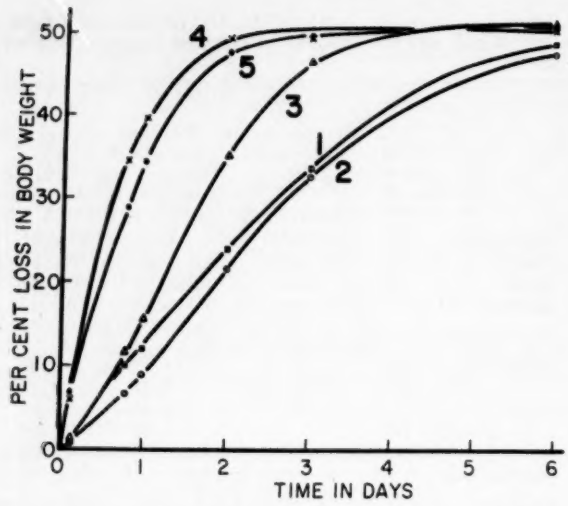

FIG. 1.—Average loss in body weight of boll weevils under desiccation after treatment: (1) Untreated live weevils, (2) untreated dead weevils, (3) dead weevils exposed to calcium arsenate, (4) live weevils exposed to calcium arsenate and then killed, and (5) live weevils exposed to calcium arsenate.

as stomach poison decreased effectiveness by one-half, indicating that these chemicals are not solely stomach poisons.

DEHYDRATION AFTER TREATMENT.—Field-collected weevils were held without food overnight and separated into lots of 50. These lots were treated as follows: (1) Untreated live weevils, (2) untreated weevils killed with cyanide, (3) weevils killed with cyanide and then exposed to calcium arsenate by gentle shaking several times inside dusted petri dishes, (4) live weevils confined for 1½ hours in petri dishes dusted with calcium arsenate and then killed with cyanide, (5) weevils confined as in (4) but not killed. The petri dishes were dusted in the chamber, three at a time, with 160 mg. of calcium arsenate. There were two lots of 50 weevils in each treatment.

After treatment, the total body weight of each lot of weevils was determined. This was found to vary from 600 to 654 mg. The insects from each treatment were then transferred to a tared cylindrical metal cage (3½ inches in diameter, 3/4 inch high, with both top and bottom covered with wire screen) that had been dried in a desiccator containing calcium chloride. Cages containing the insects were weighed with an analytical balance before being placed in the desiccators, and again after certain periods of desiccation. On an average, 5.2 mg. of the insecticide was found adhering to each lot of treated weevils; therefore, this amount was subtracted from the total weight before the loss of body weight was calculated.

All the weevils exposed in dusted dishes for 1½ hours died during 24 hours of desiccation, while the mortalities of the untreated weevils desiccated for 19, 24, and 48 hours were 17%, 22%, and 90%, respectively. The per cent losses in body weight in the different treatments are shown in figure 1. Both dead and live weevils that received no calcium arsenate (curves 1 and 2) lost weight at practically the same rate. During the first day of desiccation they lost about 10% while those treated with calcium arsenate when they were alive (curves 4 and 5) lost more than 34%. The treated dead insects (curve 3) lost weight at a rate between those of the untreated and those receiving the other treatments. The slightly higher rate compared with the untreated insects suggest that they were slightly abraded during the treatment or that the calcium arsenate adsorbed a portion of the cuticular wax, or both. This desiccative action of calcium arsenate appears to be similar to that of some inert dusts and silica aerogels (Wigglesworth 1945, Ebeling & Wagner 1959).

Effects of the different arsenates on dehydration of weevils were determined in another experiment. Petri dishes were dusted with 160 mg. of each chemical, and 60 weevils were confined for 1 hour in each of two treated dishes. They were then transferred to tared cages, weighed, and subjected to desiccation in the same way as in the previous experiment.

The losses in body weight are shown in figure 2. All the weevils treated with tricalcium arsenate and with the commercial calcium arsenate died within 24 hours. Among the check and insects treated with the other chemicals, mortalities occurring within 1-, 2-, and 5-day desiccations were as follows: Untreated check, 5%, 17%, and 91%; dicalcium arsenate, 79%, 100%, and 100%; monocalcium arsenate, 57%, 78%, and 100%, respectively. Thus calcium arsenate and tricalcium arsenate caused the fastest dehydration and were the most toxic arsenates tested. Monocalcium arsenate had the least effect on dehydration.

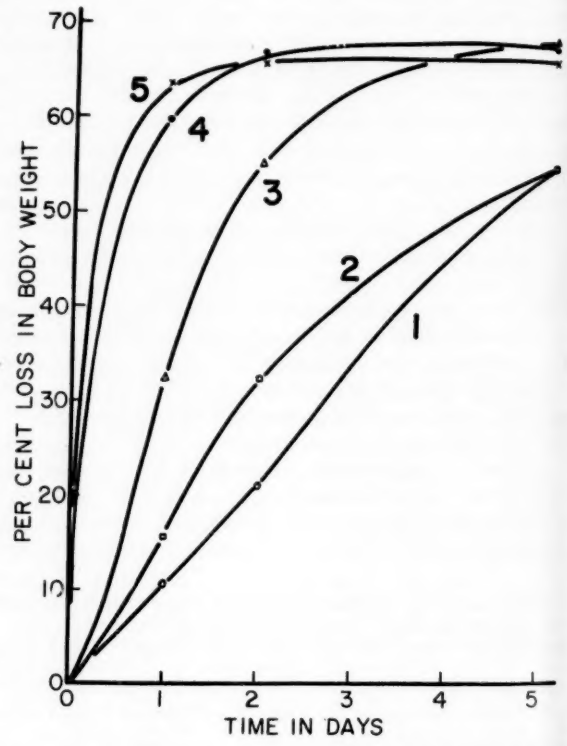

FIG. 2.—Average loss in body weight of boll weevils under desiccation after treatment: (1) Untreated check, (2) monocalcium arsenate, (3) dicalcium arsenate, (4) calcium arsenate, and (5) tricalcium arsenate.

Table 6.—Average mortality and loss in body weight of boll weevils held for 24 hours under different humidity conditions following application of calcium arsenate.

Relative Humidity (%)	Untreated Weevils Mortality (%)	Untreated Weevils Weight Loss (%)	Treated Weevils Mortality (%)	Treated Weevils Weight Loss (%)
	Series 1 (30 mg.)			
30	5.1	—	79.1	—
60	5.0	—	72.0	—
90	6.3	—	55.0	—
	Series 2 (240 mg.)			
30	7.5	18.8	100	59.4
60	2.5	15.5	100	51.4
90	2.5	11.9	70.0	21.8

EFFECTS OF RELATIVE HUMIDITY ON MORTALITY.—Two series of tests were conducted with 2-day-old square-reared weevils that had been held for 1 hour in calcium arsenate-treated petri dishes and then transferred to metal screened cages contained in covered gallon jars. The jars contained various concentrations of potassium hydroxide solutions to maintain 30%, 60%, and 90% relative humidities (Buxton 1931). In series 1, 30 mg. of the calcium arsenate were used and there were 12 replicates of 20 weevils each for the three humidity levels. In series 2, 240 mg. were used, and two lots of 20 weevils were held at each humidity.

The mortalities and loss in body weight in 24 hours are given in table 6. The mortality of treated insects decreased with increasing humidity, but the untreated insects showed little difference. The average water content of 2-day-old, square-reared weevils was found to be 76% (table 2). On this basis the weevils that had been treated with a heavy dosage of calcium arsenate lost 78%, 68%, and 29% of their water content in 24 hours at 30%, 60%, and 90% relative humidity, whereas, the untreated insects lost only 25%, 20%, and 16%.

Although it has been demonstrated that the boll weevil can be killed by the desiccating action of calcium arsenates, it is difficult to determine what proportion of the insects is killed by this action under field conditions. Data in table 4 indicate that at the two highest dosages, 40% to 48% of the dead weevils had sealed mouth parts, and presumably were killed by desiccation. Under field conditions, when the evaporation rate is higher than in the laboratory, the desiccative action of calcium arsenates may be responsible for partial control of the insects.

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Biological Degradation of *O,O*-Diethyl *O*-Naphthalimido Phosphorothioate (Bayer 22408)[1]

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Abstract

P^{32}-labeled Bayer 22408 (*O,O*-diethyl *O*-naphthalimido phosphorothioate) was converted to the oxygen analog, *O*-ethyl phosphoric, *O,O*-diethyl phosphoric, *O,O*-diethyl phosphorothioic acids and at least three other metabolites by rats, several species of insects, and cotton plants. Differences in the degradation of Bayer 22408 by the three biological systems were not the result of the number of metabolites but of the amount of each metabolite formed. Bayer 22408 was stable in insects and most of the absorbed material was recovered as administered; the oxygen analog was formed to a limited extent. Rats degraded Bayer 22408 rapidly to water-soluble phosphoric acids, which were eliminated primarily in the urine. Some Bayer 22408 escaped degradation and was eliminated intact in the feces. Bayer 22408 was not effective as an animal systemic against several ectoparasites feeding on treated rabbits. Bayer 22408 was quite stable on the foliage of cotton plants but was not translocated to untreated portions of plants.

The stability and metabolism of several phosphorothioates have been investigated in mammals and insects. When administered orally to mammals phosphorothioates are hydrolyzed rapidly and eliminated largely in the urine and to a lesser extent in the feces (Robbins *et al.* 1957, Plapp & Casida 1958b, 1958c, Krueger *et al.* 1959). Several dialkyl aryl phosphorothioates underwent *in vitro* and *in vivo* hydrolyses at both the alkyl-phosphate and the aryl-phosphate bonds (Plapp & Casida 1958c). The higher percentage of hydrolysis at the alkyl-phosphate bond by mammals as compared to insects suggests that this method of detoxification may be more important with mammals than with insects (Plapp & Casida 1958b, 1958c). In animal systemic studies, the phosphorothioates have a longer residual effectiveness against ectoparasites than phosphates (Brady *et al.* 1960). The increased residual effectiveness of phosphorothioates as compared with phosphates has been attributed to greater stability to enzymatic hydrolysis and lower volatility (Geary 1953, Ivy *et al.* 1955).

O,O-diethyl *O*-naphthalimido phosphorothioate (Bayer 22408) is a new phosphorothioate that compares favorably with the least toxic of the organophosphates to mammals and is highly effective against many species of insects (Geary 1958). The study reported herein with Bayer 22408 concerns its absorption, distribution, stability, and characterization of metabolites in white rats, cotton plants, and several species of insects.

Procedure.—*Materials.*—P^{32}-labeled Bayer 22408 was synthesized by Farbenfabriken Bayer chemists and was provided for this study through the courtesy of Vero Beach Laboratories, Incorporated. When synthesized the specific activity of the radioactive Bayer 22408 received August 15, 1958, was 2.70 mc./gm., and the specific activity of the second batch received December 8, 1958, was 1.73 mc./gm. The Bayer 22408 was purified on a Celite partition column and only one radioactive material was eluted. An infrared spectogram (Bayer 22408 in carbon tetrachloride) of the material received August 15 was identical to that of a column purified sample of nonradioactive Bayer 22408, whereas an infrared spectrogram of the radioactive material received December 8 contained an extra peak at 8.3 microns. This extra absorption band was still present after the second column purification. Bayer also provided for this study the technical grade oxygen analog, *O,O*-diethyl *O*-naphthaloximido phosphate (Bayer 25820), of the parent compound.

Column Chromatography.—The solvent system used in Celite partition chromatography (Gardner & Heath 1953) of Bayer 22408 and the oxygen analog (Bayer 25820) was acetonitrile saturated with *n*-hexane as the stationary phase and *n*-hexane saturated with acetonitrile with 4% (v/v) chloroform added as the mobile phase. Thirty ml. of the stationary phase was thoroughly mixed with 30 gm. of Celite 545 and then enough mobile solvent was added to make a slurry. The Celite slurry was packed into a chromatographic column (2.6×26 cm.) and the column was used in chromatographing 50- to 100-mg. samples of Bayer 22408 and the oxygen analog. This solvent system was adequate to separate these two closely related materials. Bayer 22408 was eluted from the column with 320 to 400 ml. of solvent, while Bayer 25820 moved off the column with 520 to 760 ml. Phosphorus-containing materials that remained on the column after using the acetonitrile-*n*-hexane-chloroform mixture were eluted with 200 ml. of methanol.

Co-chromatography on Celite (fig. 1) was used to establish the presence of metabolites of Bayer 22408 from biological systems. Samples of nonradioactive Bayer 22408 and Bayer 25820 were mixed with the chloroform-soluble radioactive materials from biological systems and placed on the chromatographic column. The identical chromatographic behavior of total phosphorus-containing (Allen 1940) and radioactive materials was used as the basis for determining the presence of residual Bayer 22408 and the biological formation of the oxygen analog and other metabolites. The amount of each P^{32}-labeled constituent was calculated quantitatively from the percentage of the radioactivity appearing in each chromatographic fraction.

An anion exchange resin (Plapp & Casida 1958a) was used in the co-chromatography of known nonradioactive *O*-ethyl and *O,O*-diethyl phosphoric and *O,O*-diethyl phosphorothioic acids with the radioactive hydrolytic products from Bayer 22408 degradation. *O*-ethyl and *O,O*-

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diethyl phosphoric acids moved off the anion exchange column with a hydrochloric acid elution gradient of pH 2–1, while the phosphorothioic acid was eluted with pH 1–0 (1:3 with methanol). This solvent system was not adequate for complete separation of *O*-ethyl and *O,O*-diethyl phosphoric acids.

Rat Metabolism and Residues in Tissues.—Mixed sexes of white rats weighing approximately 150 gm. each were treated with radioactive Bayer 22408 orally at 100 mg./kg. (3 replicates of 3 rats each), subcutaneously at 500 mg./kg. (2 replicates of 3 rats each and 3 replicates of 2 rats each), and dermally at 1000 mg./kg. (3 replicates of 2 rats each). The P^{32}-labeled material used to treat rats subcutaneously and dermally was diluted 1:4 with nonradioactive Bayer 22408 and administered in corn oil. The compound was administered orally with a polyethylene stomach tube, and the dermal application was made to a shaved area on the back of each rat.

Urine and feces from each replicate of rats were collected quantitatively (Comar 1955) following treatment at daily intervals for 7 days. Aliquots of 0.1 ml. of urine and samples of 100 mg. of feces were radioassayed, and the percentage of the total administered dose eliminated in the excreta was calculated.

The urine collected each day was extracted six times with an equal volume of chloroform, and the chloroform-soluble residue was co-chromatographed on a Celite column with nonradioactive Bayer 22408 and Bayer 25820. The water portion, containing the radioactivity which would not partition into chloroform, was regarded as hydrolytic products and was co-chromatographed on an anion exchange column with known diethyl phosphoric and diethyl phosphorothioic acids. The feces were handled in the same manner as the urine, except that they were ground into a powder with a mortar and pestle, mixed with water, and then extracted with chloroform.

Six of the rats treated orally and subcutaneously and 3 of the rats treated dermally were sacrificed 3 days after treatment, and the remaining 3 rats from the oral and dermal treatments and the 6 rats from the subcutaneous treatment were sacrificed 7 days after treatment. The total p.p.m. of radioactive materials and the total p.p.m. of chloroform-soluble radioactivity occurring in the blood, brain, heart, kidney, liver, fat, muscle, and skin were determined. The p.p.m. based on radioactivity were corrected to account for the proportionate amount of nonradioactive Bayer 22408 used in the treatments.

Insect Metabolism.—Metabolism of radioactive Bayer 22408 was studied in the boll weevil, *Anthonomus grandis* Boh.; house fly, *Musca domestica* L.; corn earworm, *Heliothis zea* (Boddie); German cockroach, *Blattella germanica* (L.); and variegated cutworm, *Peridroma margaritosa* (Haw.). Two groups of 200 insects of each species were treated topically at the rate of 25 mg./kg. using the microdrop technique. The insecticide was dissolved in acetone or 1,4-dioxane. At 1 and 4 hours after treatment, unabsorbed or excreted radioactivity was removed by surface washing the insects in acetone. The insects were then macerated in water with a Waring Blendor and the aqueous phase extracted six times with chloroform. The chloroform extracts were processed and co-chromatographed in the same manner as described for the chloroform-soluble materials from rat urine. The quantity of hydrolytic products was not sufficient for anion exchange chromatography.

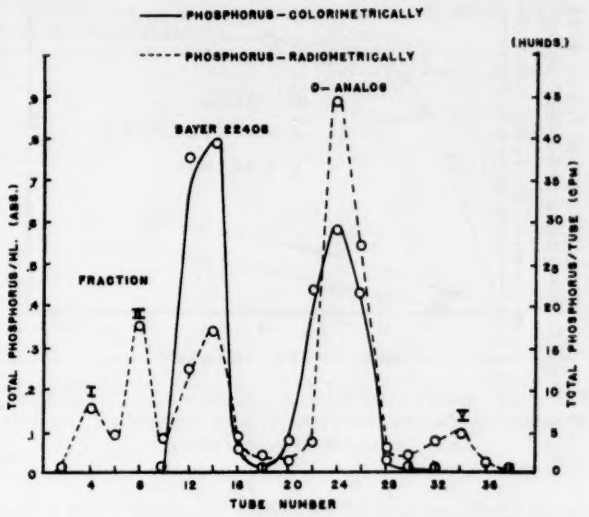

FIG. 1.—Co-chromatogram on Celite of technical Bayer 22408 and the oxygen analog (Bayer 25820) with the chloroform-soluble materials recovered from the urine of rats one day after oral treatment with radioactive Bayer 22408.

Plant Metabolism, Residues, and Translocation.—Cotton plants that were 24 to 30 inches high, fruiting freely, and growing under field conditions were sprayed (Xylene-Triton X 155-water emulsion) at the rate of 2 pounds Bayer 22408 per acre. From 50 to 75 gm. of leaves were collected at random from the plants for radioassay at 3, 7, and 14 days following treatment. The leaves were macerated in 125 ml. distilled water, and the aqueous portion was extracted six times with chloroform. The chloroform-soluble radioactivity was used to calculate the p.p.m. toxic residue remaining on or in the plants at each posttreatment time interval. The oily residue remaining after evaporation of the combined chloroform extracts was co-chromatographed on Celite, and the water-soluble portion was subjected to anion exchange chromatography.

In translocation studies, cotton plants growing under field conditions were treated with 25 mg. of radioactive Bayer 22408 at the base of each plant. The water emulsion was applied with a hypodermic syringe. Upper and lower leaf samples were collected at daily intervals for 5 days. The amount of translocation was based on total radiophosphorus, and the nature of the translocated materials was based on chloroform-water partition coefficients.

Other Studies.—*In vitro* metabolism of Bayer 22408 by rat liver slices (DuBois *et al.* 1950) was studied using radiometric and chromatographic analyses for characterizing degradation products.

In animal systemic studies, procedures were essentially those described by Adkins *et al.* (1955). Two rabbits weighing approximately 3 kg. each were treated orally at 50 mg./kg., and three rabbits were treated at 25 mg./kg. From 40 to 50 nymphs of the Gulf Coast tick, (*Amblyomma maculatum* Koch), 20 to 25 fifth-instar nymphs of the bed bug, (*Cimex lectularius* L.), and 10

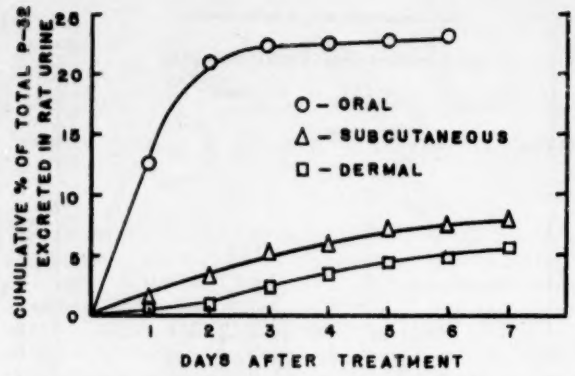

FIG. 2.—Cumulative percentage of the total administered dose of radioactive Bayer 22408 excreted in the urine of rats treated orally, subcutaneously and dermally.

to 20 adult stable flies (*Stomoxys calcitrans* (L.)) were allowed to take a blood meal from each treated rabbit. Mortality counts were made 24 hours after completion of the blood meal.

Mixed sexes of white rats (20 to 25) weighing approximately 200 gm. each were treated orally or subcutaneously with Bayer 22408 for LD-50 determination. The Bayer 22408 was administered in corn oil and rats were held under observation for 72 hours. The topical LD-50 of Bayer 22408 to 3-day-old female house flies (5 replicates of 10 flies each at each dosage level) was also determined, using standard laboratory procedures (Brady *et al.* 1960).

Bayer 22408 was subjected to bromine water (Fallscheer & Cook 1956) and peracetic acid (Tsuyuki *et al.* 1955) oxidation in an attempt to form the oxygen analog. Effectiveness of the oxidation process was measured by chromatographic and infrared analyses.

RESULTS.—*Rat Excretion, Metabolism, and Tissue Residues.*—The cumulative percentage of radioactive materials excreted in the urine from orally-treated rats was about three times greater than that from rats treated subcutaneously and about four times greater than that from rats receiving dermal treatment (fig. 2). There was little difference among the cumulative percentages of

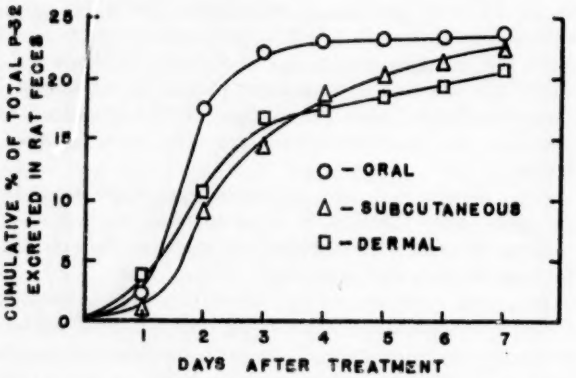

FIG. 3.—Cumulative percentage of the total administered dose of Bayer 22408 excreted in the feces of rats treated orally, subcutaneously and dermally.

radioactivity eliminated in the feces from orally, subcutaneously, or dermally treated rats (fig. 3). The percentages of the total dermal and subcutaneous doses eliminated in the feces were considerably higher than the percentage eliminated in the urine. Almost equal amounts were excreted in the urine and feces of orally treated rats (figs. 2 and 3).

The amount of radioactive hydrolytic products remaining in the urine after six extractions with chloroform was about the same for orally, dermally, or subcutaneously treated rats (table 1). A greater percentage of the radioactive materials was excreted as hydrolytic products in the urine than in the feces. More residual Bayer 22408 or unhydrolyzed metabolites were recovered from the feces of dermally treated rats than from rats treated subcutaneously or orally.

The oxygen analog of Bayer 22408 was formed quite readily by rats and eliminated in the excreta following any method of treatment (table 2). In the 3-day urine from orally treated rats, there was no residual Bayer 22408; most of the radioactivity was eluted from Celite in the methanol fraction. In the feces from rats treated orally, dermally, or subcutaneously, most of the chloroform soluble radioactivity was recovered as unchanged Bayer 22408 (table 2). There was less radioactivity in the methanol fraction from the feces than from the urine regardless of the method of treatment.

The P^{32}-labeled metabolite(s) represented by the methanol fraction was extractable from water with chloroform but was not eluted from Celite with the acetonitrile-*n*-hexane solvent. A greater percentage of the methanol fraction was present in the urine from orally treated rats than in the urine from dermally or subcutaneously treated rats. All of the materials eluted in the methanol fraction were not phosphorus-containing. Upon standing for several months the methanol fraction yielded a crystalline material that was recrystallized from *n*-hexane and methyl alcohol. An infrared spectra of this crystalline material (in Nujol) indicated a mixture of naphthalic acid and naphthaloxime (known materials provided by Bayer). There was no evidence that the naphthalic acid or naphthaloxime was conjugated with normal metabolites, since no hydrolysis was necessary to recover these derivatives.

The chloroform-soluble products of the urine and feces contained a less polar fraction than Bayer 22408 and in some instances a more polar material than the oxygen

Table 1.—Percentage hydrolysis products of radioactive Bayer 22408 excreted in the urine and feces of rats treated orally at 100 mg./kg., subcutaneously at 500 mg./kg., and dermally at 1000 mg./kg.[a]

Days After Treatment	Per Cent Hydrolysis[b]					
	Oral		Subcutaneous		Dermal	
	Urine	Feces	Urine	Feces	Urine	Feces
1	97.8	91.9	95.0	71.8	99.2	65.0
2	97.7	90.2	96.4	79.3	95.6	73.9
3	98.6	95.6	95.0	84.6	97.3	78.0

[a] Averaged from 9 rats treated orally, 12 rats subcutaneously, and 6 rats dermally.
[b] Based on chloroform: water partition distribution.

Table 2.—**Fractionation on Celite of radioactive Bayer 22408 and metabolites from several biological systems with known carrier compounds.**[a]

Biological System	Time After Treatment	Per Cent Present as: Fraction I	Bayer 22408	Bayer 25820	Fraction V	Methanol Fraction
Oral, rat urine	1 day	6.3	16.1	11.5	0.9	65.2
	2 days	6.2	9.0	53.3	.0	31.5
	3 days	1.2	.0	22.2	.0	76.6
Subcutaneous, rat urine	1 day	2.1	61.1	16.9	.0	19.9
	2 days	2.4	65.4	10.8	.0	11.4
	3 days	2.0	45.4	21.9	5.0	25.7
Dermal, rat urine	2 days	.4	45.6	29.1	.0	25.1
	3 days	.2	61.1	29.8	.0	9.0
Oral, rat feces	1 day	2.9	60.0	21.2	5.7	10.4
	2 days	5.4	53.9	30.1	.0	10.6
	3 days	4.6	51.1	7.2	.0	37.6
Subcutaneous, rat feces	1 day	3.7	68.4	13.6	.0	14.3
	2 days	12.4	50.4	24.0	.0	12.8
	3 days	5.5	71.0	21.8	.0	1.8
Dermal, rat feces	1 day	.5	88.8	9.5	.0	1.2
	2 days	.9	71.4	22.8	.0	4.9
	3 days	2.1	68.8	26.8	.0	2.3
Rat liver, *in vitro*	1 hour	.4	44.3	33.8	18.3	3.2
	4 hours	6.9	25.3	30.7	6.8	30.3
Boll weevil	1 hour	1.1	98.7	.2	.0	.0
	4 hours	5.8	84.2	.0	10.0	.0
Corn earworm	1 hour	2.2	97.8	.2	.0	.0
	4 hours	.0	100	.0	.0	.0
Cutworm	1 hour	.0	100	.0	.0	.0
	4 hours	.0	97.7	.0	.0	3.3
House fly	1 hour	1.5	86.4	4.2	.0	8.0
	4 hours	.9	83.5	13.4	1.3	.9
German roach	1 hour	1.2	98.0	.7	.0	.3
	4 hours	2.8	68.4	2.8	7.6	18.5
Cotton plant	3 days	0.1	89.2	6.8	.0	2.8
	7 days	1.8	89.3	3.1	.0	5.7
	14 days	1.2	90.5	3.2	.0	5.0

[a] Per cent Bayer 22408 and metabolites averaged from two chromatographic determinations for urine from oral and subcutaneous treatments; other percentages are from single chromatograms.

analog (Fraction I and V, fig. 1). Fraction I was actually composed of two materials (Fractions I and II, fig. 1) not well separated on the column. Fraction I had a partition distribution between *n*-hexane and 50% acetonitrile+50% water of over 100 to 1 as compared with 2 to 1 for Bayer 22408, 1 to 10 for Bayer 25820 and 1 to over 100 for Fraction V. The partition distribution ratios support evidence in table 2 that the phosphorus-containing fractions from Celite were different materials. Fraction V was produced more readily *in vitro* by rat liver slices than *in vivo* by rats.

Anion exchange co-chromatography of the hydrolytic products in the urine and feces from rats yielded *O*-ethyl and *O,O*-diethyl phosphoric and *O,O*-diethyl phosphorothioic acids with the latter constituting the predominant hydrolytic product (table 3). There were only minor differences between the ratios of each acid formed following any route of administration; however, there were more *O*-ethyl and *O,O*-diethyl phosphoric acids on the first day following treatment of rats orally or subcutaneously than on the second and third days after treatment.

Toxic residues (based on chloroform-soluble radioactivity) remained in the kidney and muscle for a longer period of time when rats were treated subcutaneously than when treated orally or dermally (table 4). All radio-

Table 3.—**Hydrolytic products of radioactive Bayer 22408 from rat excrements.**[a]

Biological System	Days After Treatment	Per Cent Present as— $C_2H_5OP(O)(OH)_2$ +$(C_2H_5O)_2P(O)OH$	$(C_2H_5O_2P(S)OH$
Oral, rat urine	1	35.4	64.6
	2	20.3	79.7
	3	17.1	82.9
Subcutaneous, rat urine	1	31.9	68.1
	2	25.3	74.7
	3	21.8	78.2
Dermal, rat urine	1	21.2	78.8
	2	21.1	78.9
Oral, rat feces	1	22.8	77.2
	2	23.8	76.2

[a] Per cent of total radioactivity using an anion exchange resin with identifications based on co-chromatography; duplicate determinations for urine from oral and subcutaneous treatments with single determinations for dermal treatment.

active materials in the blood, brain, fat, and heart were in the form of hydrolytic products at 3 days after treatment. Although there was a rapid disappearance of the residues from the skin of dermally treated rats between 3 and 7 days following treatment, the skin still contained about 40 p.p.m. toxic residues after 7 days. The liver and kidney contained the highest amounts of total radioactive materials, but only a small portion of these materials were chloroform-soluble.

Insect Metabolism.—The chloroform-soluble radioactivity recovered from insects was largely unchanged Bayer 22408, as determined by Celite partition co-chromatography (table 2). The oxygen analog was formed in small amounts by all insects but was formed more readily by the house fly and the German cockroach. A less polar fraction than the parent compound was recovered from most of the insect species and was always eluted from Celite in conjunction with the heaviest concentration of insect pigments. Also, a more polar fraction than the oxygen analog was present in boll weevils, house flies, and German cockroaches. The identity of these two fractions from insects was not established, but these unidentified materials exhibited the same chromatographic behavior as the unknown fractions recovered from rat urine and feces.

Cotton Plant Metabolism, Residues, and Translocation.—Bayer 22408 was quite stable on cotton plants treated at the rate of 2 pounds technical per acre. Three days following treatment, about 60% of the total radioactive materials were chloroform-soluble products, while total P^{32}-labeled residues were 194 p.p.m. At 7 days following

Table 4.—**P.p.m. radioactive Bayer 22408 and derivatives in tissues of rats treated orally at 100 mg./kg., subcutaneously at 500 mg./kg., and dermally at 1000 mg./kg.**

Tissue[a]	P.p.m. Chloroform-Soluble Materials: Oral 3 Days	Oral 7 Days	Subcutaneous 3 Days	Subcutaneous 7 Days	Dermal 3 Days	Dermal 7 Days
Kidney	0.31	<0.01	1.10	0.35	< 0.01	< 0.01
Liver	.94	< .01	1.05	.32	2.22	1.44
Muscle	< .01	< .01	.56	.31	< .01	< .01
Skin	< .01	< .01	28.84	16.97	269.60	39.75

[a] The blood, brain, fat, and heart contained less than 0.01 p.p.m. at the 3- and 7-day sacrificing intervals for all routes of administration.

treatment, there were 19 p.p.m. chloroform-solubles and 126 p.p.m. based on total radioactivity while at the 14-day time interval the values were 10 p.p.m. and 93 p.p.m., respectively. When fractionated on a Celite column, 90% of the chloroform-soluble radioactivity was recovered as unchanged Bayer 22408 (table 2). Some oxygen analog was formed with the greatest percentage occurring in the leaves analyzed at 3 days after treatment. Fraction V that was isolated from rat excreta and insects was not present in cotton plants.

Only 5% of the Bayer 22408 applied to the base of cotton plants was translocated. There was more radioactivity translocated to the leaves on the first day following treatment than during the remainder of the 5-day experimental period. Unchanged Bayer 22408 accumulated in the lower basal leaves during the first 24 hours after treatment. As the Bayer 22408 was degraded, more radioactive materials accumulated in the top leaves. By 5 days after treatment, all translocated radioactive materials were in the form of hydrolytic products, as determined by chloroform:water partition coefficients.

Toxicity Studies.—In animal systemic studies with Bayer 22408, one rabbit treated orally at 50 mg./kg. died about 2 hours after treatment, while another rabbit exhibited symptoms of severe poisoning. There was no mortality of bed bugs or stable flies that fed on the rabbits at 1 hour after treatment. Bayer 22408 was apparently nontoxic to three rabbits treated orally at 25 mg./kg., and there was no mortality of bed bugs, stable flies, or Gulf Coast ticks that fed on the treated animals.

The acute oral LD-50 of Bayer 22408 to mixed sexes of white rats was 335 mg./kg., while the subcutaneous LD-50 was greater than 1000 mg./kg. Rabbits were considerably more susceptible to oral treatments with Bayer 22408 than rats. Neither naphthalic acid nor naphthaloxime was toxic to rats when administered orally at 2000 mg./kg. The topical LD-50 of Bayer 22408 to the house fly was approximately 10 mg./kg.

Chemical Oxidation.—When the chloroform-soluble materials recovered from bromine water or peracetic acid oxidation of Bayer 22408 were fractionated on Celite, two phosphorus-containing fractions were recovered. Fraction A from both bromine water and peracetic acid oxidation of Bayer 22408 yielded a spectrogram identical to the parent material, while Fraction B was unlike Bayer 22408 or the oxygen analog. Fraction B contained absorption bands at 7.75 and 10.25 microns, which were not present in the spectrogram of Bayer 22408, while Bayer 22408 had a peak at 15.45 microns corresponding to the P=S group that was not present in Fraction B. A spectrogram of the oxygen analog contained absorption bands at 7.96, 8.25, and 14.95 microns that were not present in the spectra of Fraction B. Neither bromine water nor peracetic acid was an effective oxidizing agent for converting the phosphorothioate to the phosphate structure.

DISCUSSION.—The *in vivo* metabolic pathway of Bayer 22408 (table 2) proceeds through enzymatic oxidative and hydrolytic reactions. The same group of metabolites was isolated from rats, insects, and plants, but the percentage of each metabolite formed was vastly different for the three organisms. Fraction I (fig. 1 and table 2) may have resulted from a complete degradation of Bayer 22408 to phosphoric acid and then incorporated into a normal metabolite that was less polar than the parent material. Some pigments from rat excreta, insects, and plants were extractable from water with chloroform and were eluted from Celite before Bayer 22408; Fraction I was always eluted in conjunction with the highest concentration of pigments.

Insects, plants, and rats converted the phosphorothioate to the phosphate or oxygen analog (table 2), which is usually the effective antiesterase agent. The occurrence of more oxygen analog in the chloroform-solubles from rat excreta as compared with insects does not coincide with toxicity data, since Bayer 22408 was only moderately toxic to rats and highly toxic to the house fly. This lack of correlation may be attributed to the greater stability of Bayer 22408 in insects as compared with its rapid degradation in rats.

Fraction V and the methanol fraction (table 2) recovered from the three different biological systems may represent "desethyl" Bayer 22408 or "desethyl" Bayer 25820, which would result from hydrolysis of an *O*-ethyl ester linkage of the parent compound and the oxygen analog, respectively. Such a detoxification mechanism has been used to explain the selective toxicity of such compounds as Dipterex (Robbins *et al.* 1956, Arthur & Casida 1958) and ronnel (Plapp & Casida 1958b).

The occurrence of more *O*-ethyl and *O,O*-diethyl phosphoric acid on the first day following treatment of rats orally or subcutaneously, as compared with smaller amounts present on the second and third days after treatment, was unusual, as compared with other phosphorothioates (Plapp & Casida 1958b, Arthur & Casida 1959). The high percentage of hydrolytic products present in the excreta indicated that Bayer 22408 was rapidly degraded regardless of the route of administration. The appearance of more radioactivity in the feces than in the urine of dermally or subcutaneously treated rats was also unexpected but was substantiated by treatment of additional replicates of rats with the second shipment of Bayer 22408. These differences in excretory patterns cannot be attributed entirely to contamination of the feces with the urine or in the case of dermal treatments to ingestion of the applied material. These findings may have practical significance in the control of livestock pests that breed in feces.

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A Versatile Automatic Microapplicator[1]

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ABSTRACT

An automatic microapplicator has been built which is suitable for administering either topical or injected doses of toxic solutions or suspensions. A time delay relay controls the delivery of a wide choice of volumes ranging from approximately 0.5 to 5 microliters with a tolerance of 5% or less, using a 0.25-ml. glass syringe and a number 27 hypodermic needle. The details of construction and operation are described.

The urge to obtain quantitative information from chemical treatment and biological response experiments has resulted in the development of mechanical devices which facilitate the injection or topical application of chemical solutions in amounts and with an accuracy consistent with the experimental requirements. Busvine (1957) and Metcalf (1958) have thoroughly reviewed the published techniques of insecticide microadministration. Other devices which were not cited in these reviews include those described by Brezner (1959), Chao & Ball (1956), and Shambaugh (1952).

Most microapplicators provide adequate precision and replication using small volumes (*e.g.*, 0.1 to 10 μl.) containing various amounts of chemicals. Mechanized models permit one to conduct a large number of tests in a relatively short time. The automatic microapplicator described below meets not only these criteria but offers a simple and versatile adjustment of the delivery volumes.

DESCRIPTION.—The general placement of parts in the microapplicator is shown in figure 1. A finished machine which was designed by the senior author and constructed by the Iowa State University Instrument Shop is illustrated in figure 2. Descriptions of the parts and their functions follow. The time delay relay,[2] which begins its cycle in response to pressure on a foot switch, starts and stops the clock motor. At the end of a preset time interval, the time relay automatically resets. It is necessary to actuate the foot switch for each delivery, thereby eliminating possible unintentional multiple deliveries. Power is supplied to the gear drive by a clock-type synchronous motor[3] which starts and stops instantly, thereby eliminating variations in the amount of rotation for a given time interval. The clock motor advances the micrometer barrel during administration of solutions or retracts the micrometer barrel in preparation for another series of applications. The micrometer shaft moves in and out of the hollow end of the rotating drive shaft. A set screw in the drive shaft bears on a groove cut in the micrometer shaft.

Three bevel gears of equal diameter and two sets of reset and advance gears (each set with a 3:1 speed reduction ratio) are arranged so that the turning micrometer advances the syringe plunger with the transmission reverse knob pushed in. When the transmission reverse knob is pulled out the motor returns the micrometer barrel to the starting position. The brass reduction gears have a one-eighth inch face, 64 pitch, and 20° pressure angle.[4] The syringe pedestal has a spring-loaded release bar designed to permit easy release of the syringe. Base plate (14″×6″), housing (5″ high), and pedestal are aluminum.

The upper portion of the electrical circuit (fig. 3) controls the clock motor; the lower portion of the figure shows the time delay relay circuit. The latter is built into the relay and is joined to the rest of the circuit through

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[2] Cramer Controls Corp., Centerbrook, Conn., type 412-E, 15-second range, wiring diagram #1121, 115 volt, 60 cycle.

[3] Cramer Controls Corp., type 117-P, 12 RPM, 115 volt, 60 cycle.

[4] Boston Gear Works, Quincy 71, Mass.

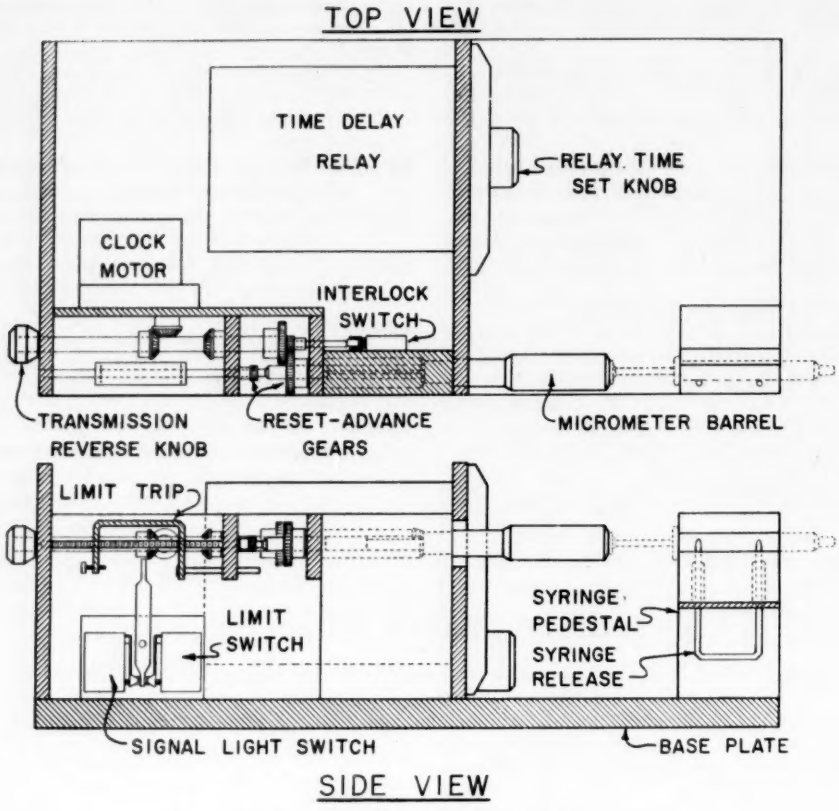

FIG. 1.—Schematic representation of the microapplicator.

the terminal connections indicated by numbers. Miniature, single pole, single throw, roller-actuated microswitches are used for the limit, signal light, and interlock switches. The limit and signal light microswitches are actuated by the same lever and remain set in one direction until moved to the opposite position by a tripping mechanism. A screw drive for the tripping mechanism is an integral part of the micrometer drive shaft.

The three switches of the syringe circuit (fig. 3) are shown set for delivery. With the limit switch moved to the opposite direction, power can not be applied to the clock motor until the transmission reverse knob is pulled out. This allows the interlock switch to move in the opposite direction to that shown in the diagram so that power again goes to the motor. When the micrometer has returned to the limit of rewind or starting position, the limit switch is again tripped. Power can not be applied to the clock motor until the transmission shaft is pushed in and the interlock switch moved to the advance position. The signal light portion of the syringe circuit indicates that the apparatus is ready for delivery (green light on); or that the micrometer has advanced to its farthest position (red light on) and needs resetting.

PERFORMANCE.—Operation of the microapplicator is very simple. A syringe is calibrated to determine the average delivery for different time intervals. An actual set of calibration data for a 0.25-ml. tuberculin syringe equipped with a 27-gauge hypodermic needle is shown in table 1. In our case, solutions of radioactive insecticides in acetone also provide confirmatory calibration data. From these data, a straight line can be fitted to a plot of delivery times versus volumes delivered. The exact time required to deliver a desired volume can then be selected. This time interval is set on the time delay relay, and trial measurements and adjustments are made to obtain exact delivery.

After the syringe has been filled with an appropriate solution (*e.g.*, acetone solution of an insecticide) actuation of the foot switch is all that is necessary for each delivery. After the micrometer reaches the limit of its forward travel, the transmission shaft is pulled out and the micrometer returns to the starting position. The resetting process takes about 60 seconds; during this interval the

Table 1.—Calibration (with mercury) of a 0.25-ml. syringe equipped with a 27-gauge hypodermic needle.

TIME INTERVALS (SEC.)	AVERAGE MERCURY DELIVERED[a] (mg.)	(μl.)	DELIVERY VARIATION (%)
1.5	6.3	0.47	4.0
3	13.1	0.97	4.6
6	26.2	1.94	3.0
9	39.4	2.91	2.3
12	52.4	3.87	2.6
15	65.6	4.85	1.8

[a] Based on 10 consecutive deliveries at each time interval; temperature of mercury 25° C.

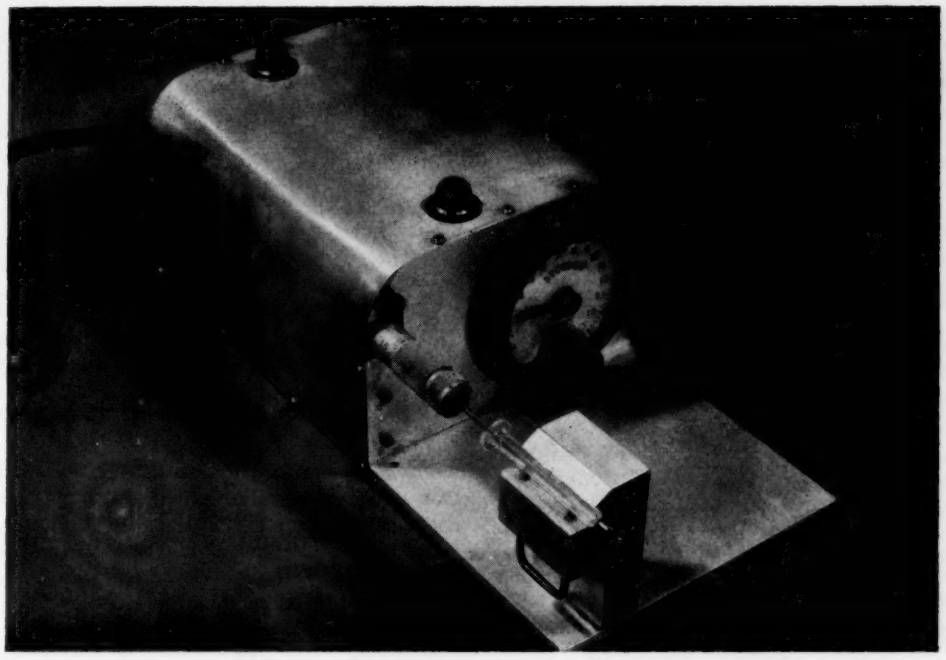

FIG. 2.—Three-quarter view showing the time delay relay dial and syringe pedestal.

syringe can be removed and refilled with another solution. The barrel of the micrometer can be returned easily to the starting position from any intermediate position by tripping the limit switch with a bypass button and pulling out the transmission shaft.

A 0.25-ml. tuberculin syringe and number 27 gauge hypodermic needle commonly used in this type of microapplicator will result in the delivery of approximately 5 microliters with one revolution of the micrometer barrel (the micrometer advances 0.025 of an inch). A 3 to 1 gear reduction from the motor to the micrometer barrel gives a speed of 4 RPM (1 revolution of the micrometer barrel in 15 seconds) with a 12 RPM motor. Therefore, a time delay relay with a maximum range of 15 seconds and a synchronous clock motor with a speed of 12 RPM (one revolution in 5 seconds) were selected to give the required operations. Once the time delay relay has been set for a given delivery, it has a repeatable accuracy of 0.5% of full scale deflection, or 0.075 second. In terms of delivering 1μl. the relay provides a theoretical accuracy of 0.025 μl., which is far greater than most experiments require. Greater precision can be achieved through the use of syringes with smaller diameters and leakproof plungers.

The microapplicator has been used mostly to make topical applications of acetone solutions of insecticides to the mesonotum of house flies, *Musca domestica* L. With the syringe and needle described above, a house fly is conveniently treated with 1 μl. of insecticide solution. One house fly bioassay design we have been following consists of treating duplicate groups of 50 female flies for each of four doses (100 flies per dose); this design is used for series of both test and standard preparations. Under these conditions, we are treating 800 flies or more a day at an average rate of 10 flies per minute including time for changing solutions in the syringe. It has been found that application of acetone solutions of DDT with this microapplicator has given better regression lines of transformed per cent mortality on log dose than were obtained with a manually operated microapplicator.

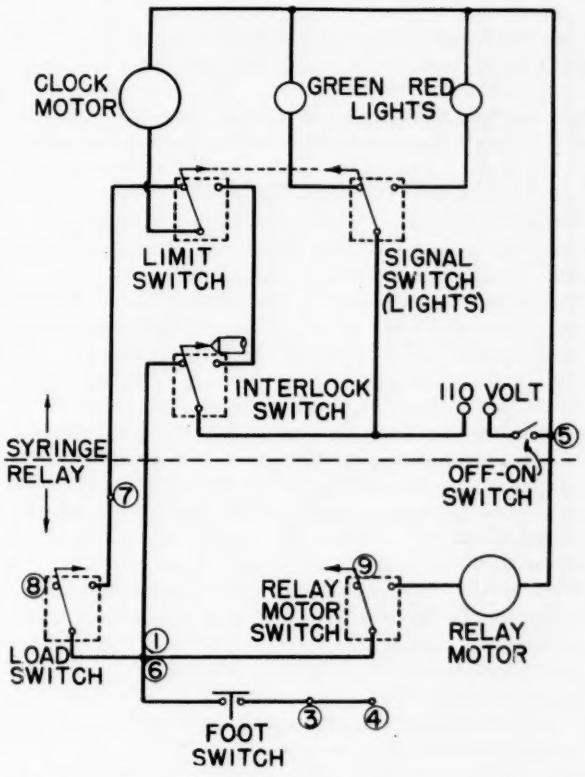

FIG. 3.—Schematic diagram of the microapplicator electrical circuit.

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Performance of Sevin Against Grape Berry Moth[1]

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ABSTRACT

Sevin®(1-naphthyl methylcarbamate) wettable power at a concentration of 2 pounds of the 50% per 100 gallons proved to be as effective as the standard, DDT, for control of grape berry moth, *Paralobesia viteana* (Clem.). At a 1-pound rate, the control was somewhat inferior to 2 pounds. By cholinesterase assay, Sevin was found to be compatible with several low soluble copper compounds and the fungicide, Phaltan® (N-trichloromethylmercaptophthalimide) but incompatible with copper fungicide mixtures containing hydrated lime. In the presence of lime, Sevin was completely hydrolyzed within 60 minutes. Sevin deposits on the fruit were reduced 32% to 40% in 7 days and by 85% or more within 4 weeks. The amount of residue found in juice was similar to that found on the fresh fruit.

Recent investigations have shown DDT, parathion and EPN to be highly effective in the control of the grape berry moth, *Paralobesia viteana* (Clem.), the major fruit-infesting pest of vineyards in the Chautauqua Grape Belt of New York. For satisfactory results, three sprays of one of the above chemicals are necessary, two for the first brood and one for the second (Taschenberg 1953). In spite of the effectiveness of these chemicals, however, each in some way has at least one disadvantage for commercial use. Thus, three applications of DDT may leave a harvest residue in excess of the legal tolerance. Such a residue presents no problem where the fruit is to be processed into juice because virtually all of the DDT remains on the skins and is eliminated in the pomace (Taschenberg & Avens 1949). However, this DDT residue is objectionable on grapes sold as fresh fruit or where the crop is processed into products containing the skins. One means of overcoming an excessive DDT harvest residue is to substitute an organic phosphate insecticide in the third spray. Although a schedule of two DDT sprays and one spray of either parathion or EPN has given as good results as three DDT sprays, this practice has failed to be accepted generally by the growers. The majority apparently would prefer not to use a phosphate insecticide because of the safety precautions which must be observed in mixing and applying such sprays.

One of the chief aims of recent control studies is to find an effective as well as an acceptable substitute for DDT in the final spray. Many of the recently developed insecticides have been evaluated but, in this paper, an account is given of the results obtained with Sevin® (1-naphthyl methylcarbamate). The objectives in this study were: (1) how effective is Sevin, actually and relatively, for the control of the grape berry moth, (2) is it compatible with fungicides and other ingredients commonly occurring in grape spray mixtures, (3) how persistent are Sevin spray deposits and (4) how much of the chemical, if any, will occur in juice prepared from Sevin-treated grapes.

CONTROL EXPERIMENT.—The insecticides used in this investigation were applied in the form of sprays by means of a hooded boom. A detailed description of this equipment is presented in another publication (Taschenberg 1948). The spray schedule followed was two first-brood and one second-brood spray, the first application being made immediately after blossoming, the second usually 10 days later and the third in August. For the first and second sprays, the rate of application was 250 gallons per acre and, for the third, 300 gallons. An effort was made to keep the rate of application uniform by maintaining a constant rate of sprayer-speed when applying treatments.

All sprays applied to grapes in the area usually contain a fungicide as well as an insecticide together with some supplement to spread and stick the pesticides. The suggested fungicide schedule is ferbam in the first grape berry moth spray followed by low soluble copper and lime in the second and third applications. In some seasons, depending on severity of certain diseases, ferbam may be used in the first two sprays. In 1957 tests, ferbam was used at a 1.5–100 rate in the first application and low soluble copper at a 2–100 plus lime at 4–100 rate in the second and third applications. An emulsible oil was added at the rate of 8 ounces per 100 gallons where ferbam was present but at a one-quart rate in low soluble copper and lime mixtures. During 1958 and 1959, Sevin was applied in combination with the experimental fungicide, Phaltan® (N-trichloromethylmercaptophthalimide) 2 pounds per 100 gallons in all applications. With the standard insecticide, DDT, the fungicide schedule was the same as used in 1957. The sprays of Sevin plus Phaltan contained a commercial spreader-sticker, 4 ounces per 100 gallons, rather than an emulsible oil. The combination of oil and Phaltan caused some foliage injury.

All plots were four rows wide and in each row there were at least 15 vines. In all test blocks, there were two or more

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replicates of each treatment. Counts to evaluate treatments were made during harvest when 10 vines were selected about the center of the two middle rows of a plot. Twenty clusters were selected from each vine used to obtain an infestation record. The selection of clusters was made with these restrictions: 10 clusters were taken from each half of the vine, six were picked from the upper part and four from the lower part. These clusters were removed from the vines to facilitate the examination of berries for injury. On the treated clusters only the infested berries were counted; both injured and uninjured berries were counted in control clusters. The cluster is the basic unit used in calculating the results and is based on the average count in untreated clusters.

During 1957 a comparison was made of several schedules of Sevin and DDT, the results of which are presented in table 1. In this test, Sevin in a three-spray schedule proved markedly inferior to DDT. Two DDT applications followed by one of Sevin gave fair control but it was inferior to schedules of either three DDT sprays or two DDT sprays plus one of parathion. The difference in control between the schedule of two DDT sprays plus one Sevin spray and the schedule of three Sevin sprays was undoubtedly caused mostly by the control provided by DDT in the first and second sprays. As was learned later, the explanation of the poor results obtained with Sevin in this test was the presence of lime in the second and third sprays. Evidence in support of this statement was the finding that immediately after application a deposit of 2.4 p. p. m. of Sevin was found from a spray of a 1–100 rate plus 2 ounces of a spreader-sticker, whereas only 0.5 p. p. m. was found when a like concentration of Sevin was combined with copper and lime. The indication was that Sevin is affected adversely by this fungicidal mixture. Further consideration is given this point in another section of this paper.

Data obtained in 1958 and 1959 with schedules of Sevin, DDT and Guthion®(*O,O*-dimethyl *S*-(4-oxo-1,2,3,-benzotriazin-3-(4*H*)-ylmethyl) phosphorodithioate) are given in table 2. In both test blocks, three sprays of Sevin in combination with Phaltan proved to be as effective as a like number of sprays of either DDT or Guthion. Sufficient data are on record to establish that Guthion performs as well as DDT for control of the grape berry moth. Sevin was compared at two concentrations with DDT in two vineyards during 1959. The control at the 2-pound rate was similar to that obtained in 1958. At the 1-pound rate, it was inferior to the higher concentration, markedly so in one test.

In other trials made during 1958 and 1959, schedules of two DDT sprays followed by one of Sevin, Guthion or parathion were evaluated. The results from these tests are presented in table 3. As might be expected, two first-brood sprays of DDT failed to give satisfactory control. The

Table 1.—The performance of Sevin and of other materials in grape berry moth control where a low soluble copper fungicide and lime mixture was used in the second and third treatments, 1957.

Material and Pounds per 100 Gallons	Number Applications[a]	Per Cent	
		Infested	Control
Sevin, 50% (w.p.), 2.0	3	7.3	59
DDT, 50% (w.p.), 1.5	3	1.1	94
DDT, 50% (w.p.), 1.5	2	2.8	85
Sevin, 50% (w.p.), 2.0	1		
DDT, 50% (w.p.), 1.5	2	1.2	93
Parathion, 15% (w.p.), 1.5	1		
Untreated		18.3	

[a] Applications made 6/20, 7/1 and 8/5.

Table 2.—Control of grape berry moth with a schedule of three sprays of Sevin and Phaltan, 1958 and 1959.

Material and Pounds per 100 Gallons	Infested (%)	Control (%)
1958 Test 1[a]		
Sevin, 50% (w.p.), 2.0	0.6	96
DDT, 50% (w.p.), 1.5	0.6	96
Untreated	14.1	
1958 Test 2[b]		
Sevin, 50% (w.p.), 2.0	0.5	96
DDT, 50% (w.p.), 1.5	0.5	96
Guthion, 25% (w.p.), 1.5	0.3	96
Untreated	14.8	
1959 Test 1[c]		
Sevin, 50% (w.p.), 1.0	11.7	52
Sevin, 50% (w.p.), 2.0	1.2	96
DDT, 50% (w.p.), 1.5	1.0	95
Untreated	24.3	
1959 Test 2[d]		
Sevin, 50% (w.p.), 1.0	1.9	91
Sevin, 50% (w.p.), 2.0	0.8	96
DDT, 50% (w.p.), 1.5	1.1	95
Untreated	21.6	

[a] Applications made 7/1, 7/10 and 8/18.
[b] Applications made 7/2, 7/18 and 8/14.
[c] Applications made 6/23, 7/1 and 8/31.
[d] Applications made 6/24, 7/2 and 8/6.

Table 3.—Control of grape berry moth with two sprays of DDT for first brood and one spray of Sevin for second brood, 1958 and 1959.

Material and Pounds per 100 Gallons	Number Applications	Infested (%)	Control (%)
1958 Test 1[a]			
DDT, 50% (w.p.), 1.5	2	1.9	94
Sevin, 50% (w.p.), 2.0	1		
DDT, 50% (w.p.), 1.5	3	0.8	98
DDT, 50% (w.p.), 1.5	2	10.7	68
Untreated		32.7	
1958 Test 2[b]			
DDT, 50% (w.p.), 1.5	2	0.7	98
Sevin, 50% (w.p.), 2.0	1		
DDT, 50% (w.p.), 1.5	2	0.4	99
Parathion, 15% (w.p.), 1.5	1		
DDT, 50% (w.p.), 1.5	2	5.7	83
Untreated		33.1	
1959[c]			
DDT, 50% (w.p.), 1.5	2	2.5	91
Sevin, 50% (w.p.), 2.0	1		
DDT, 50% (w.p.), 1.5	2	2.0	92
Guthion, 25% (w.p.), 1.5	1		
DDT, 50% (w.p.), 1.5	3	3.6	87
Untreated		23.8	

[a] Applied 6/29, 7/23 and 8/19.
[b] Applied 6/29, 7/23 and 8/19.
[c] Applied 6/24, 7/2 and 8/6.

Table 4.—Compatibility as measured by the stability of cholinesterase inhibition effected by Sevin.

Material and Pounds per 100 Gals.[a]	Per Cent Decrease in Anti CHE Activity After Storage at 37° C. for			
	5 Min.	60 Min.	12 Hrs.	24 Hrs.
Sevin, 2	0	0	0	0
+Phaltan, 2	0	0	0	0
+Cupravit, 1	0	0	0	0
+Copper A, 2	0	0	0	0
+lime, 1	71	100	—	—
+lime, 1+Cupravit, 1	74	100	—	—
+lime, 1+Copper A, 2	68	100	—	—

[a] Sevin, 50% (w.p.); Phaltan, 50% (w.p.).

results obtained in 1959 with schedules of two DDT sprays and some different insecticide in the third application were somewhat inferior to those obtained in 1958. However, where either Sevin or Guthion was employed, both were slightly superior to a three-spray DDT program.

Compatibility Tests.—The deleterious effect of the copper-lime fungicidal sprays on the performance of Sevin has been pointed out. Because of this experience, laboratory evaluations were designed to determine if the innate insecticidal properties of Sevin were adversely affected in mixture with the various fungicidal preparations.

The fact that Sevin is an anticholinesterase (Haynes *et al.* 1957) provides a convenient means of measuring its compatibility with other compounds. This *in vitro* activity can be directly correlated with the insecticidal capacity of Sevin. The results of a series of assays on a mixture of Sevin and a given pesticide that produce a constant level of cholinesterase inhibition over a period of time suggest that the insecticidal activity will not be adversely influenced by the presence of the adjuvant. Conversely, in a case where the original inhibition level established by the Sevin is altered, and increasing enzyme activity occurs with successive assays, the combination is incompatible. The insecticidal value of such mixtures will also diminish with time.

Identity of the materials tested and the rates used are presented in table 4. A 100-ml. sample of each formulation was prepared (singly and in mixture with Sevin) in deionized water and agitated in a temperature-controlled bath maintained at 37° C. One-ml. aliquots of each preparation were periodically removed, diluted to 10 ml., and immediately assayed for anticholinesterase activity. Endogenous inhibition caused by the individual pesticides was measured simultaneously. The initial concentration of Sevin was adjusted to elicit less than 100% inhibition of the standardized house fly (*Musca domestica* L.) head enzyme brei. The enzyme preparation and manometric assay techniques used in these experiments have been described in detail (Moorefield & Tefft 1958).

In the present studies, the original base level of cholinesterase inhibition effected by the Sevin was 75%. A negligible endogenous inhibition was caused by the other materials when they were assayed individually. No alteration of enzyme activity was experienced in mixture with Phaltan, with Copper A (tetra copper calcium oxychloride) or with Cupravit (tribasic copper sulphate) (table 4), indicating that Sevin is compatible with the organic fungicide, Phaltan, and with the various copper salts. Sevin was rapidly degraded in the presence of lime, and the addition of copper to such mixtures did not noticeably retard the breakdown. As the 1-naphthyl methylcarbamate is susceptible to alkaline hydrolysis (Haynes *et al.* 1957), such results are not surprising, and these observations confirm the findings made under field conditions.

Sevin Deposits and Residues.—An important phase of the investigation evaluating Sevin for grape berry moth control was the studies to determine the persistence of deposits on fruit. Samples of fresh fruit were taken for Sevin analysis from plots treated in the same way as those plots used in control studies. Duplicate samples of berries were taken for each treatment. Each sample contained 300 berries taken from 20 vines and the same vines were used for this purpose throughout the sampling period. Three clusters were selected on each vine and five berries were cut from a cluster with shears. At each sampling, a new series of clusters was selected. Additional details on the procedure used in sampling are given in another publication (Taschenberg & Avens 1960). Because of the difficulty encountered in stripping lots of 300 berries for analyses, it was necessary to subsample. Two aliquots of 100 grams each were taken from a 300-berry sample. Each aliquot represented from 1/6 to 1/9 of the total weight of the original sample. Extraction of Sevin was carried out for a period of 5 minutes in a Waring Blendor using chloroform as the solvent. The determinations were made according to the method developed by Miskus *et al.* (1959). The recovery of insecticide by this method was 80%.

Data are given on the persistence of Sevin deposits on Concord grapes in tables 5 and 6. In general, there is a rather close agreement between deposits found on subsamples taken from the original 300-berry samples. Similar amounts of Sevin were found on fruit treated with either three sprays of Sevin or two sprays of DDT fol-

Table 5.—Disappearance of Sevin deposits from Concord grapes, 1958.

Time After Final Application (Days)	Deposit (p.p.m.)[a]					Per Cent of Original	Rainfall (Inches) Accumulated
	Sample A[b] Subsamples[c]		Sample B[b] Subsamples[c]		Average		
	1	2	1	2			
DDT, 50% (w.p.), 1.5 lbs. Applied 7/4 and 7/17 Sevin, 50% (w.p.), 2.0 lbs. Applied 8/27							
0	10.4	12.8	11.0	12.4	11.7	100	0.00
7	7.0	7.4	6.7	6.8	7.0	60	0.28
14	3.4	3.6	1.8	2.0	2.7	23	3.22
28	1.3	1.4	1.0	1.3	1.3	11	5.69
Sevin, 50% (w.p.), 2 lbs. Applied 7/4, 7/17 and 8/27							
0	9.0	9.1	10.7	12.5	10.3	100	0.00
7	7.0	9.0	5.9	6.0	7.0	68	0.28
14	3.2	3.8	1.8	2.6	2.9	28	3.22
28	1.5	1.9	1.5	—	1.6	15	5.69
Sevin, 50% (w.p.), 4 lbs. Applied 7/4, 7/17, 8/27 and 10/2							
0	27.3	31.0	30.5	25.0	28.5	100	0.00
1	24.5	27.0	24.5	26.5	25.6	90	0.00
2	25.3	23.5	20.0	22.0	22.7	80	0.00
4	22.0	23.0	22.5	—	22.5	79	0.00
7	12.5	19.2	20.0	15.5	15.5	59	0.00

[a] Apparent residue on untreated, 0 p.p.m.
Recovery of known amounts added to untreated sample 80%; values as given uncorrected for recovery.
[b] 300 berries.
[c] Weight 100 grams; contained 32 to 45 berries.

Table 6.—Residues of Sevin on fresh grapes and in juice.

Sample No.	Dates Sprays Applied	Time After Final Spray to Harvest (days)	Sevin Residue (p.p.m.)[a]					
			Fruit[b]			Juice		
			a	b	Average	a	b	Average
1	7/4, 7/17, 8/27	49	0.7	1.0	0.9	0.9	0.7	0.8
2	7/4, 7/17, 8/27, 9/23	21	3.0	2.9	3.0	2.8	2.6	2.7
3	7/4, 7/17, 8/27, 10/2	14	4.6	3.2	3.9	3.2	2.9	3.1

[a] Apparent residue on untreated 0 p.p.m.
Recovery of known amounts added to untreated sample 80%; values as given uncorrected for recovery.
[b] 300-berry sample, subsampled for analysis.

lowed by one spray of Sevin. At a concentration of 2 pounds of Sevin per 100 gallons, the deposits were from 10.6 to 12 p. p. m. on samples taken immediately after the final spray application. Within 1 week, the loss in deposit was from 32% to 40%. After 4 weeks, the loss was from 85% to 89% of the original. Of significance is the fact that the above findings on loss of deposit within 7 days after application are substantiated by the results from determinations on fruit treated with a higher concentration of Sevin applied in a schedule of four sprays (table 5). From the data shown in table 5, it appears that the factor of weathering, excluding rainfall, appears to be responsible for much of the reduction in Sevin deposits within 7 days. The disappearance of Sevin deposits on fruit in 1958 is shown graphically in figure 1.

Along with the foregoing studies, the possibility of the occurrence of Sevin in juice was investigated. In this connection, four lots of grape clusters, one untreated and three treated, were processed into juice. Each lot weighed approximately 30 pounds and was made up of clusters picked at random from 10 vines. A sample of 300 berries was obtained from each lot before processing. Five berries were cut from each of 60 clusters, these clusters having been selected at random from the 30-pound sample. In general, the procedures followed in preparing the juice closely simulated those followed in commercial practice, the exception being the removal of berries from stems by hand rather than by a stemming machine. The results of the analyses are given in table 6. It is obvious from these data that the amount of Sevin found in the juice closely approximates that found on the pre-processed fruit.

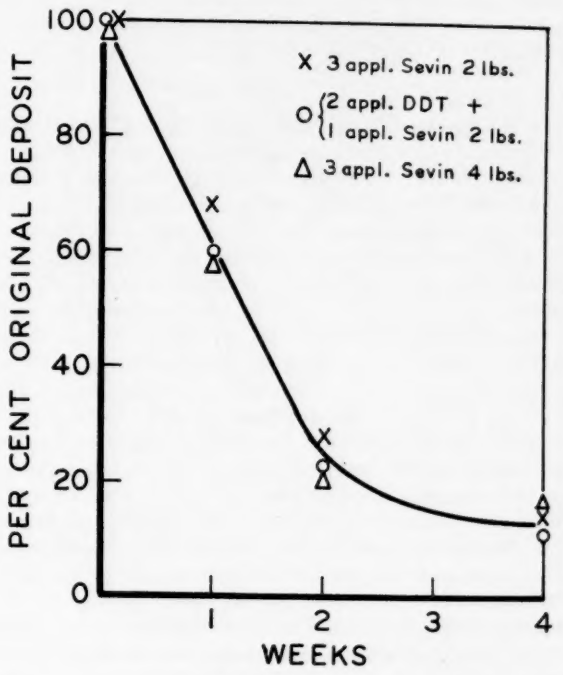

FIG. 1.—Disappearance of deposits of Sevin on Concord grapes, 1958.

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Grub Population in Turf Varies with pH Levels in Ohio Soils[1]

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Abstract

A state-wide turf grub and soil acidity survey was conducted in Ohio from 1954 through 1958. These studies indicate that the Japanese beetle, *Popillia japonica* Newm., increases in numbers more rapidly in low pH soils and that a high grub population continues in these soils year after year as long as favorable weather prevails during the egg and young larval stage. A similar behavior is indicated for the grubs of the northern masked chafer, *Cyclocephala borealis* Arrow, whereas the grubs of the June beetles, *Phyllophaga* spp., seem to reach their maximum accumulation at a pH of about 6.0.

In the investigation of the relationship of soil pH and the population of grubs of the Japanese beetle, *Popillia japonica* Newm., it has been shown by Wessel & Polivka (1952) and Polivka (1960) that it is possible to reduce the level of grub population by simply applying lime to soil. To gain more specific evidence on the relationship between soil acidity and grub population, a state-wide pH and grub survey was initiated to determine whether such a difference occurs naturally throughout the infested areas in Ohio. These surveys which were conducted over a period of 5 years (1954–1958) took into consideration the grub population of the northern masked chafer, *Cyclocephala borealis* Arrow, and the June beetles, *Phyllophaga* spp., as well as the Japanese beetle. Turf areas in cemeteries, golf courses, playgrounds, and parks were used for this study. However, most of the data reported on herein was obtained from cemeteries because it was found that most cemeteries had received little or no lime or fertilizer.

Procedure.—In setting up the survey procedure, the aim was to obtain both pH and grub population data from one or more turf areas in each county in the State. However, in 1954, a majority of the 235 locations checked were taken in the southern and eastern counties. In succeeding years, two or more areas were sampled for pH and grub population from each county.

Soil for pH determinations was obtained by taking 10 cores three-fourths of an inch in diameter and 1 inch in length between the 1- and 2-inch level. These samples were then airdried at room temperature, crushed, and screened and checked for soil acidity expressed as pH values.

Grub population data for the 1954, 1955, and 1956 records were obtained by examining the soil from 10 cores 8 inches in diameter to the depth of 4 to 10 inches, depending upon the depth at which larvae were found, in each area sampled. In 1957 and 1958 the grub population was determined by examining the soil from 10 to 25 four-inch cores for each area. The number of cores examined was determined by the size of the area under observation.

Results.—When the soil acidity data were grouped into three pH levels of 4.9 and lower, 5.0 to 5.9, and 6.0 and higher, it was found that the east-northeastern section of the State had pH values of 4.9 and lower. The north-central, central, and southeastern area had a pH of 5.0 to 5.9, except for a small section at the junction of Licking, Muskingum, and Perry Counties. The western one-half of the State had a pH of more than 6.0, except for small areas in Cincinnati and Toledo (fig. 1).

Results of these surveys are shown graphically in figures 2, 3, 4, 5, and 6. The solid line which is marked with a J represents the average grub population of the Japanese beetle for the various soil acidity levels, the broken line marked with a C represents the average grub population of the northern masked chafer, and the line marked with a P represents the average grub population of the June beetles.

Figure 2 shows the result of the survey conducted in the fall of 1954 and the spring of 1955. This graph shows that the greatest Japanese beetle grub populations were found in soils with a pH of 4.9 or lower. It also shows that the grub population per square foot decreased as the soils approached a pH of 7.0 or neutrality. The population of the northern masked chafer followed approximately the same slope in the line except at a much lower average number per square foot. On the other hand, the June beetle population was found to be the lowest in the low pH soils and increased in numbers as the soil approached the point of neutrality.

The graphs that show the results of the 1955–56, 1956–57, 1957–58, and 1958–59 surveys were similar to those shown in figure 2. In comparing these graphs with each other, it is found that there was an increase in the Japanese beetle grub population each year over that of the preceding year for each of the first 3 years of the survey, whereas in each of the last 2 years the population declined. These differences in the grub population are especially true in the most acid soils. The cause of the reduction in population levels was undoubtedly owing to moisture conditions that were unfavorable for the development of the insect.

Rainfall, according to Hawley (1949), is one weather factor that is largely responsible for the fluctuation in grub population from one year to another. The amount of rainfall occurring during the period when the eggs and young larvae are present in the soil, which is generally during the month of July, largely determines the density of the fall grub population. In 1957, the moisture condition in July was slightly toward the dry side for insect development as compared with conditions existing in July of 1954, 1955, and 1956 (U. S. Weather Bureau records for years 1954 to 1958). This effect was of such proportion as to cause a slight reduction in the 1957 fall grub population. The lack of sufficient rainfall undoubtedly affected the hatching of the eggs and establishment of the young larvae.

In 1958, the average population of the Japanese beetle grubs in all areas sampled was found to be two grubs per square foot less than that found in 1957. It was also found that in 1958 the State as a whole had an average July rainfall of 7.89 inches. In unpublished laboratory data, Wessel (1951) reported that, as the amount of water added to soil in rearing boxes containing Japanese beetle eggs increased, the per cent survival of newly hatched

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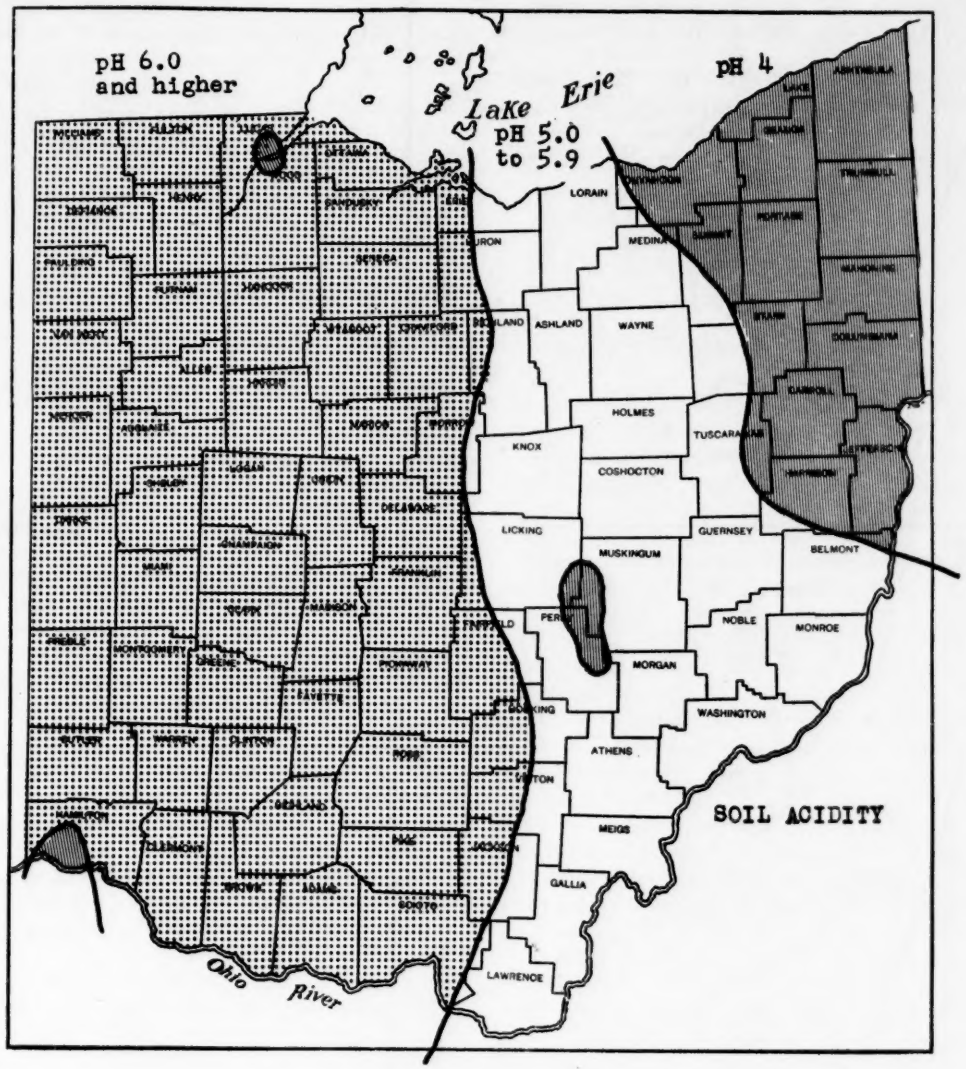

FIG. 1.—Soil acidity map of Ohio.

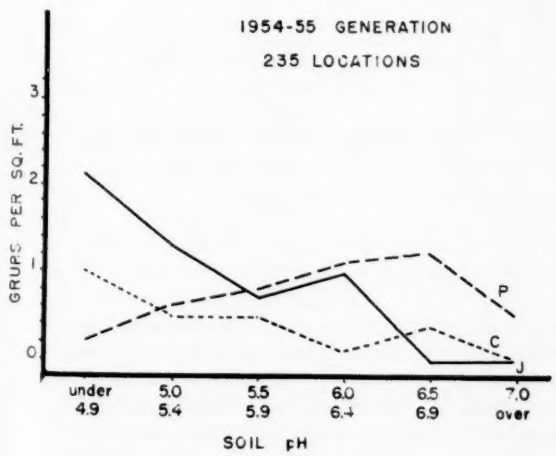

FIG. 2.—Grub population in relation to soil acidity for 1954–55 generation.

grubs decreased. The lowest survival occurred in the boxes that received a maximum 1.50 inches of water at single weekly applications. Therefore, a reduction of the Japanese beetle grub population over the State in 1958 was not surprising. However, in a few areas, where the rainfall was near average for July, there was an increase in the grub population over that found in 1957.

While rainfall in the month of July has a decided effect upon the Japanese beetle grub population, rainfall in June apparently is one of the determining factors in the northern masked chafer grub survival. According to the data in figures 2, 3, 4, 5, and 6, the increasing grub population from 1954 to 1958 would indicate that favorable rainfall conditions prevailed during the egg and young larval stages of the northern masked chafer.

The graphs also indicate that grubs of the June beetles are most generally found in soils with a pH of around 6.0. These findings agree fairly well with those of Hammond (1948) in Ontario. Serious turf damage has been found to occur largely in areas where the grass has been mowed

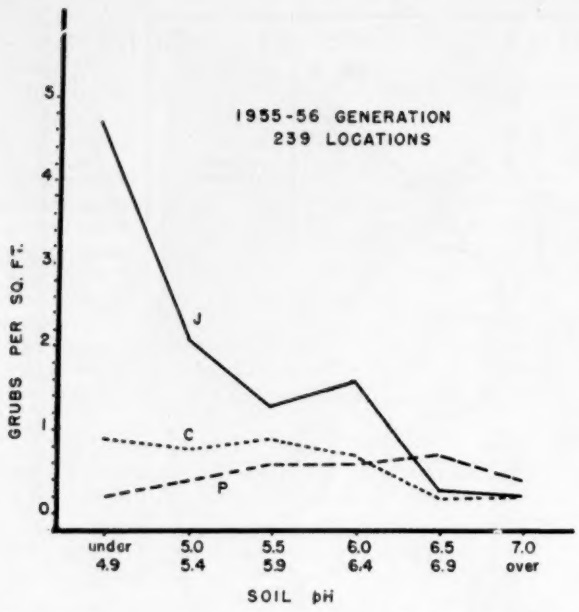

FIG. 3.—Grub population in relation to soil acidity for 1955–56 generation.

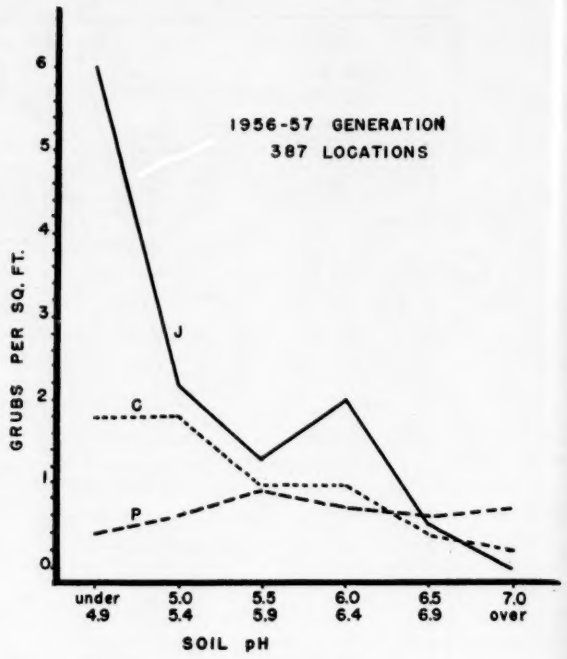

FIG. 4.—Grub population in relation to soil acidity for 1956–57 generation.

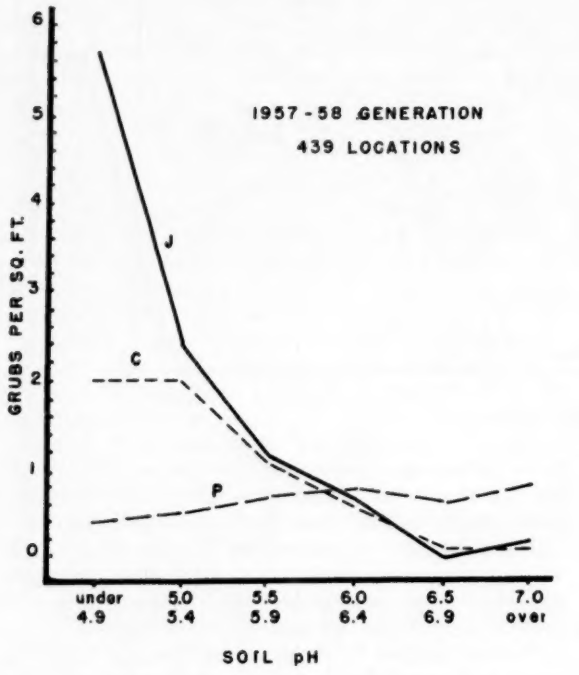

FIG. 5.—Grub population in relation to soil acidity for 1957–58 generation.

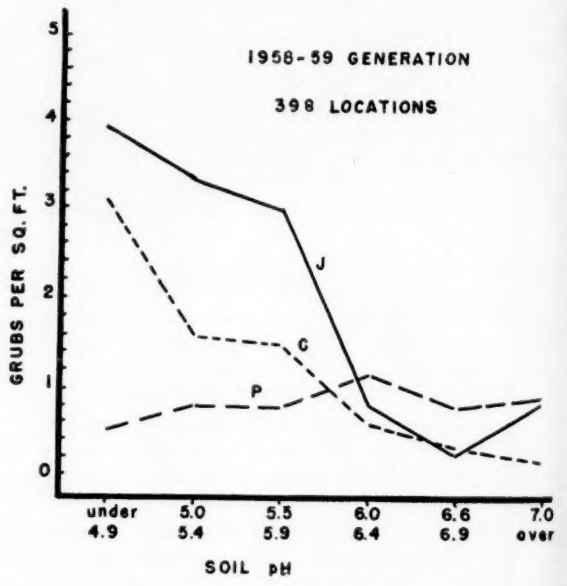

FIG. 6.—Grub population in relation to soil acidity for 1958–59 generation.

close on soils with a pH of 6.0 to 7.0, which corresponds favorably with the findings of workers who have studied June beetle infestations in heavily grazed pastures (Graber *et al.* 1931). Very few *Phyllophaga* grubs have been found in Ohio in soils with a pH of less than 5.0 to 5.4.

In summarizing the results of this study, the data indicate that the long-time behavior of turf grubs, especially grubs of the Japanese beetle, is influenced by soil acidity. In the low pH soils, the effect is shown by the more rapid increase in numbers of Japanese beetle grubs and the persistence of a high grub population year after year as long as favorable weather prevails during the egg and young larval stage. A similar behavior is indicated for *Cyclocephala* grubs, whereas *Phyllophaga* grubs seem to reach their maximum accumulation at a pH of about 6.0.

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Resistance of Dollard Red Clover to the Pea Aphid, *Macrosiphum pisi*[1]

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ABSTRACT

Plants of the Dollard variety of red clover, *Trifolium pratense* L., were more resistant to the pea aphid, *Macrosiphum pisi* (Harris), than plants of the Wegener variety. Resistance in Dollard was owing to nonpreference and antibiosis. This resistance to aphids appeared to explain a much lower incidence of virus in Dollard than in Wegener when these varieties were grown under field conditions at St. Paul, Minnesota. When the mosaic and pea stunt viruses were inoculated mechanically, Dollard was just as susceptible as Wegener. Breeding red clover for resistance to aphids may be a more successful approach than trying to breed for resistance to virus diseases.

Field observations have indicated repeatedly that the Dollard variety of red clover (*Trifolium pratense* L.) is less likely to become infected with the clover virus complex present in Minnesota than the Wegener variety (Thomas & Wilcoxson 1960). In the fall of 1958, for example, plots of Wegener at St. Paul had more than 90% of the plants infected with mosaic, while adjacent plots of Dollard had less than 10% infection. The question arose as to whether the lower incidence of the virus in Dollard might be the result of resistance to virus infection or owing to klendusity, as illustrated in the resistance of raspberry to the aphid, *Amphorophora rubi* (Kalt.), (Rankin 1927, Schwartze & Huber 1937, Huber & Schwartze 1938). Work on this problem was started through laboratory research projects in a course on "Insects in Relation to Plant Diseases" taught by the authors at the University of Minnesota.[3]

Jewett (1941) reported differences in resistance to the pea aphid among red clover varieties. He found that a strain of red clover from Minnesota had significantly higher counts of aphids than other strains of red clover from Kentucky, Tennessee, and Virginia. Furthermore, there were differences in resistance among plants within a given strain. Varietal resistance to the pea aphid has been reported also in alfalfa (Blanchard & Dudley 1934, Painter & Grandfield 1935), in peas (Maltais 1936), and in vetch (Albrecht 1940). The general subject has been reviewed by Painter (1951).

MATERIALS AND METHODS.—Seedlings of Dollard and Wegener red clover were grown in flats in the greenhouse and when plants were about 1½ months old they were transferred to pots containing a 3:1 mixture of loam and sand. When the potted plants were growing vigorously, but not yet in bloom, they were exposed to the pea aphids. All of the work was done in greenhouses where air temperatures averaged approximately 70° F.; however, there was a daily variation from about 65° to 80° F.

The pea aphids were obtained originally from alfalfa in the field and were reared in the greenhouse on broad beans (*Vicia faba* L.). Supplementary light was supplied over each rearing cage to give a 16-hour day.

Fifteen different trials were completed on the relative attractiveness of Dollard and Wegener. In each trial five plants of each variety were selected on the basis of uniformity of vegetative characteristics and were exposed to 50 to 400 pea aphids. The insects were permitted to select plants at random. In some trials petioles were drawn across a runway so that the insects released on the runway had a free choice between Dollard and Wegener plants. In other trials potted plants were arranged in a circle, and the insects were placed in a petri dish in the center of the circle. The insects then had to crawl from the dishes, across the table, and up the pots before finding the desired host. Location of the aphids was determined usually 24 to 36 hours after the tests began, but in a few trials this was done after 1 week.

In three experiments known numbers of aphids were confined on Dollard and Wegener plants for periods of 15 to 22 days in order to observe the increase in the population. Observations were made also on the longevity of individual aphids and numbers of progeny produced by single aphids on plants of each variety. Because aphid mortality was high when the insects were transferred from broad beans to red clover, the aphids for this particular experiment were obtained from heavily infested red clover plants.

RESULTS.—Pea aphids preferred Wegener over Dollard in all experiments. An average of 75% of the aphids selected Wegener rather than Dollard when given a free choice. In one of the 15 trials, 54% of the insects selected Dollard; however, most of the aphids were on a single plant which had white leaf markings, a characteristic not typical of Dollard. In all of the other trials, from 62% to 90% of the insects selected Wegener. Frequently Dollard plants were free from insects throughout the tests; all

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Table 1.—Numbers of *Macrosiphum pisi* produced on plants of Dollard and Wegener red clover during periods of 15 to 22 days.

Trial No.	Days of Confinement on Clover	Aphids/5 Plants When Trial Began		Aphids/5 Plants When Trial Ended	
		Dollard	Wegener	Dollard	Wegener
1	20	20	22	70	580
2	22	5	5	25	315
3	15	22	23	458	495
Average		16	16	184	463

Table 2.—Reproductive life span of individual females of *Macrosiphum pisi* and the number of nymphs produced on Dollard and Wegener red clover.

Dollard		Wegener	
Life Span (days)	Nymphs Produced	Life Span (days)	Nymphs Produced
9	23	9	47
2	1	4	20
2	3	2	6
4	1	17	88
4	5	6[a]	44
4	10	4[a]	20
9	15	7	37
Average 5	8	9	37

[a] Insect still alive but test was terminated.

Wegener plants had some aphids on them. Insects which selected plants of Wegener usually began to feed with a minimum of restlessness, but insects which selected plants of Dollard began to feed only after considerable restless activity or they frequently left the plant without feeding.

Some limited work was done to investigate possible mechanisms involved in the host preference. In one test the possible influence of odor was investigated with an olfactometer containing chopped leaves and petioles of Dollard and Wegener plants. Insects were allowed to move about in the olfactometer for 5 hours and then their position in relation to the chopped tissues was determined. There was no apparent attraction of the insects by odors from the plant tissues, for the aphids wandered aimlessly in the olfactometer without coming to rest over the plant tissues.

An attempt was made also to alter the attractiveness of Dollard and Wegener plants by painting plants of one variety with a water extract of leaves and petioles of the other variety. These painted plants were then exposed to aphids. As in the other tests, the Wegener plants were more attractive than the Dollard plants.

The pubescence on leaves and petioles of Dollard and Wegener was examined in a further search for a possible explanation for the difference in host preference, but no differences in pubescence were found.

Following confinement of a known number of aphids on Dollard and Wegener for 15 to 22 days, the resulting populations were larger on Wegener than on Dollard (table 1). In the first and second trials the aphid populations were 8 to 15 times greater on Wegener. In the third trial the difference was not large and may have been caused by inadvertent selection of some Dollard seedlings which were susceptible to the aphids.

Observations on individual aphids indicated that the life span of mature aphids was possibly somewhat shorter on Dollard than on Wegener, but it was relatively short on both varieties (table 2). However, the individual aphids on Wegener produced four times as many nymphs as those on Dollard.

DISCUSSION.—The Dollard and Wegener varieties of red clover differed markedly in their resistance to the pea aphid. The resistance of Dollard was apparently owing to both nonpreference and antibiosis. Both of these types of resistance have been reported in other host plants of the pea aphid (Blanchard & Dudley 1934, Harrington 1941, Cartier 1959). Dahms & Painter (1940) reported antibiosis in some selections of alfalfa and tolerance in others.

Although the majority of Dollard plants appeared to be resistant to aphids, a number of susceptible plants were observed. Jewett (1941) observed differences in susceptibility to pea aphid among plants within a variety of red clover, while similar observations on alfalfa were reported by Dahms & Painter (1940). Such differences can be expected because varieties of red clover consist of plants with different genetic makeup.

The lower incidence of virus observed in the Dollard variety in the field appears to result from its resistance to the pea aphid and is therefore believed to be an example of klendusity. In tests with mechanical inoculations in the greenhouse, Dollard appeared to be just as susceptible to the mosaic and pea stunt viruses as Wegener. Breeding red clover for resistance to aphids may be a more successful approach than trying to breed for resistance to the several different virus diseases. A similar conclusion was reached by Knight *et al.* (1958) with respect to the control of virus diseases of the red raspberry.

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Incidence of Root-Feeding Weevils, Root Rot, Internal Breakdown, and Virus and Their Effect on Longevity of Red Clover[1]

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ABSTRACT

Root growth of red clover and injury by the clover root curculio (*Sitona hispidula* (F.)), the weevil *Calomycterus setarius* Roelofs and the clover root borer (*Hylastinus obscurus* (Marsh.)) and their association with root rot, internal breakdown of crown tissue, and virus were investigated by weekly examinations of plants during two growing seasons. The data indicate that weevil damage, internal breakdown, and associated and independent root rot are the major factors in the lack of persistence of red clover. The interaction of these factors probably contributed to the arrested plant growth during June of the second year and at the end of October had killed two-thirds of the plants in the experimental plot.

Most stands of red clover in the northeastern United States decline rapidly during their second year, and only a few plants survive the second winter. Stand loss has been variously attributed to root-feeding insects, root rot, and virus infection, either separately or in combination. Although red clover often behaves as a biennial in the northeastern States, it has many characteristics of a perennial. Buller & Garza T. (1959) report on an excellent performance of red clover in Mexico under five harvests the first year and six harvests the second year. Smith (1950) found that root reserve fluctuations in red clover resembled those in perennial alfalfa rather than the pattern occurring in biennial sweetclover. Red clover has been maintained into its third year in our greenhouse when cut at the bloom stage. In order to determine what factors actually kill plants and in what sequence, red clover plants were dug weekly from a field at University Park, Pennsylvania and examined for root size; for injury caused by the clover root curculio (*Sitona hispidula* (F.)) and the clover root borer (*Hylastinus obscurus* (Marsh.)); and for rot occurring separately or associated with injury. The presence of foliar virus symptoms and any other factors that might affect longevity were recorded. One factor that proved to be important was the presence of larvae of the weevil, *Calomycterus setarius* Roelofs, which resembles the immature *Sitona*, and likewise feeds on rootlets and the root surface of red clover. Another was an internal breakdown occurring within the crown-root area of many plants (Graham *et al.* 1960). Its development and incidence were traced in the plants along with insect and root rot injury.

PREVIOUS STUDIES.—Many important phases covering the biology of the clover root borer (Rockwood 1926), the clover root curculio, (Wildermuth 1910, Bigger 1930, Marshall & Wilber 1934), and *C. setarius* (Johnson 1944) have been studied and reported. Likewise, a number of plant pathologists have studied the root rots and viruses affecting red clover (Kreitlow *et al.* 1953). The factors are briefly reviewed here as background information for a better understanding of the studies reported here.

Root-Feeding Weevils.—Adult clover root borers spend most of their lives within red clover roots, where they tunnel as they feed and make galleries for their eggs. The hatching grubs in turn make additional tunnels as they feed and grow and in which they eventually pupate and transform to adults. In the spring, adult borers leave the roots for a short flight period which terminates in their infesting new red clover fields.

Clover root curculio adults emerge in late spring from pupae formed within cells made in the soil, and from then until late fall infest new fields by walking, for although they have wings they make no use of them. Eggs are dropped on the soil by wandering adults in September and October. The hatching larvae enter the soil where they feed on red clover rootlets, and when larger, severely gouge and scour the outer surface of large roots, often to a depth of several inches. They pass the winter in the adult stage, also as eggs and possibly as small larvae. Oviposition is resumed in the spring.

Calomycterus setarius weevils emerge late in June from pupae formed in the soil around several plants including clovers. Adults are wingless and infest new fields by walking. They lay eggs in clusters in soil or debris in midsummer and die in the fall. Hatching larvae enter the soil and feed on roots and organic matter. The immature larvae resume activity in early spring and continue feeding to early June.

All these weevils have but one generation each year.

Root Rots, and Viruses.—Root rot is one of the principal factors limiting persistence of stands. Several different organisms may attack the roots successively and these become involved in a root-rot complex. Among the fungi most commonly associated with root rots of red clover are the species *Fusarium oxysporum* Schlect., *F. solani* (Mart.) Appel & Wr., and *F. roseum* (Lk.) Snyd. & Hans. The tap roots of diseased plants are found to be light brown to black when cut open. The rot may be localized or extend throughout the central core of the root. The tap roots of dying plants are almost always completely rotted.

Several red clover viruses are widely distributed throughout the United States. One of the most common is red clover vein mosaic. Red clover is also a host of bean

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yellow mosaic and potato yellow dwarf virus, and other viruses normally occurring in alfalfa, peas, and white clover can also infect and seriously weaken or kill red clover.

MATERIALS AND METHODS.—On April 25, 1958, a 50-×100-foot plot adjacent to weevil-infested Ladino white clover and red clover, to insure an adequate insect infestation, was seeded broadcast to Pennscott red clover. Starting on July 9, and continuing weekly to October 15, 1959 (except from December 3 to March 19), 25 plants and their root systems were dug at random. Roots were washed and examined visually for curculio feeding injuries, for external rot occurring separately or associated with this injury. The incidence of virus was based on foliar symptoms only. Since it is impossible to identify viruses on this basis, identifications were not attempted. The roots were cut longitudinally at the crown-root area and their diameters measured. They were then inspected for internal breakdown, and for borers or their tunnels. These weekly data were summarized on a monthly basis for the 15-month period.

A limited number of additional studies were conducted at the field plot simultaneously to obtain information on additional phases of insect and rot activity. One such study required the erection of a 4-×16-foot migration board painted on both sides with a sticky material to trap flying root borers. Its daily examination supplied data on the duration and intensity of adult flights for comparison with the invasion of red clover roots. In addition 1-square-foot surface litter samples were taken at random to determine the adult *Sitona* and *Calomycterus* population, and similar samples to a depth of 6 inches to determine larval populations and thus reveal their trends and peak attack on the root system. Ninety-five per cent of the *Sitona* obtained as adults or reared from larvae collected in the plot and adjacent red clover were identified as *hispidula*. The others were *flavescens*.

RESULTS.—Root growth and the incidence of root borer and weevils, rot, internal breakdown, and virus infection for each month during the first and second crop years are shown in table 1. Trends of these factors are briefly reviewed for significant deviations and possible relationships.

Root Growth.—In 1958 average root diameters increased from 4.6 mm. in July to 7.2 mm. in October and remained rather constant until December. In 1959 they continued to enlarge from 8.4 mm. in March to 12.8 mm. in September and showed a slight decline in October. The trend shows a period of retarded growth in June 1959 that according to our weekly data actually extended from May 28 to June 18.

Clover Root Borers.—There were almost no borer-infested roots during the first year even though an adjacent 2-year-old field was heavily infested. Daily removal of adults from the sticky migration board showed the flight period was May 2 to July 2, with greatest activity from May 13 to 18. Just why these adults did not infest the plants in the experimental field is not known.

Adults trapped in 1959 showed a flight period from May 5 to June 10, with greatest activity from May 6 to 24. The first adult borer in the experimental plot was found on May 7, and the incidence of infestation attributed to flights reached 35% in June. The further increase to 53% in August suggested that there was considerable plant-to-plant movement after the flight period.

Table 1.—Monthly[a] root growth and incidence of weevils, root rot, internal breakdown, and virus in red clover during 1958 and 1959.

Month	Average Root Diameter (mm.)	Per Cent of Plants Showing					
		Borers	Rot with Curculio-*Calomycterus*	Rot with Curculio-*Calomycterus*	Total Rot	Internal Breakdown	Virus
1958				*Seedling Year*			
July	4.6	0	6	—	12	12	—
August	6.4	1	9	3	19	23	—
September	6.6	0	22	7	24	23	12
October	7.2	0	45	26	37	18	5
November	7.1	2	43	19	33	17	9
December	7.3	0	44	12	28	32	0
1959				*Second Year*			
March	8.4	0	54	22	42	32	—
April	9.2	0	53	25	53	49	4
May	10.3	7	56	33	69	70	12
June	9.9	35	85	53	97	81	20
July	11.9	51	86	78	100	81	43
August	12.3	53	91	84	99	78	40
September	12.8	46	89	76	100	76	50
October	11.6	30	86	74	98	66	48

[a] All monthly figures based on the average of 4 samples of 25 plants each taken at weekly intervals except December (1 sample), March (3 samples) and October 1959 (2 samples).

Clover Root Curculio and Calomycterus.—From 6% to 45% of the first-year plants showed external feeding, which peaked in October and remained constant into December. By March 1959 the incidence had increased to 54%. This level was 56% in May, increased to 85% in June, and remained rather constant thereafter.

Examination of litter samples in August 1958 indicated that the new adult levels were 1 curculio and 1 *C. setarius* per square foot. Most of these adults probably migrated into the field, but the July incidence suggested that a few larvae survived seeding operations and developed in the field.

Random soil samples taken in May 1959 gave *C. setarius* larval levels of 11 per square foot, and in early June curculio and *C. setarius* larvae averaged 27 and 15 per square foot, respectively. Thus a total of 42 larvae per square foot were feeding externally on the roots. This peak attack occurring from May to mid-June coincided with the flight borer invasion.

Root Rots.—Total rot, at first superficial, affected 12% of the clover roots at their first examination when only 12 weeks old. This incidence increased to 37% in October. It can be seen in comparing the seedling year curculio-*Calomycterus* injured root samples with and without rot that about one-third were associated with rot in August and this figure increased to 58% in October. Most of the discoloration in the chewed areas was shallow and confined to the cortex of the root.

In the second year total rot climbed rapidly from 42% in March to 100%in July. Again by calculation, it may be noted that during this period rot developed in 41% to 92% of the chewed areas, reaching a climax in August. This relationship between curculio and borer injury and rot substantiates previous greenhouse studies (Graham & Newton 1959).

It is probably significant that the period of greatest acceleration in total rot likewise coincides with the root borer flight and the curculio-*Calomycterus* attack in June 1959.

Internal Breakdown.—This disorder was present in 12% of the clover plants but not necessarily those with rot when first examined at the age of 12 weeks. It appeared as a small dark area in the pith of the crown. The per cent of infested plants increased to 32 in December, and the necrotic areas also increased in size. In 1959 the incidence increased from 32% in March to 81% in June and July.

A number of necrotic areas expanded to the exterior after midsummer and, in conjunction with insect damage, outside root rot and the progression of fungi down old cut flower stalks, caused a complete breakdown of the crown and upper tap root. However, internal breakdown appeared arrested in 17% to 24% of the plants during July to October. In these plants the light brown, mostly dry tissue became walled off by healthy tissue. Simultaneously 15% to 17% of the total plants formed a second crown above the internal breakdown area, and from August to October a few plants having new crown tissue developed a second internal breakdown.

Greatest acceleration of internal breakdown occurred in April and May 1959. The expanding necrotic areas were avoided by the invading and tunneling borers. They were found to invade surrounding tissue, and in doing so opened the way for more root-rotting organisms.

Red Clover Viruses.—A count of virus-infected plants was not started until September 1958, when 12% were affected. From April to July in the second year incidence increased to 43% then leveled off in August.

Weakened and Dead Plants.—About 20% of the plants sampled in August 1959 had been killed by the combined factors, and in September dead plants in the sample jumped to 40%. By November three-fourths of the original stand was severely weakened or dead. Many of the weakened plants were wholly dependent upon adventitious roots for their survival. It became increasingly difficult to assess cause of damage in the weakened, rotting, and dead plants in September and October. For that reason the study was discontinued in October.

DISCUSSION.—The data suggest that any one of the major factors—weevil damage, root rot, or internal breakdown—may seriously limit the maintenance of red clover stands. The relationship between weevil injury and rot increased their destructiveness. The sequence and pattern of the various factors and the incidence of injury are important considerations in evaluating their potential for destroying red clover.

In the first sampling the percentages of plants with rot and internal breakdown were higher than those of plants showing curculio-*Calomycterus* injury. Curculio-*Calomycterus* injury surpassed both in October and remained high into December. During the winter both curculio-*Calomycterus* injury and total rot increased, rot more so than weevil injury, while internal breakdown remained unchanged.

From March to May of the second year total rot and internal breakdown continued to increase but curculio-*Calomycterus* injury remained constant until May-June larvae reached both full growth and peak numbers. The rather high incidence of these three factors was further augmented by the additional stress produced when borers invaded the roots. Borers did not use the necrotic internal breakdown or rot areas, but attacked the greatly reduced and probably weakened living tissue surrounding them and extending into the roots. The destruction of tissue by internal breakdown, rot ,weevils, and borers decreased the roots' ability to function and to store reserve foods. The damage by these overlapping attacks was further augmented by an increasing incidence of virus infection. These combined factors coincided with the May 28 to June 18, 1959 period of retarded growth and from July through October weakened and killed about two-thirds of all red clover plants.

The apparent decrease in incidence of weevils, associated rot, and internal breakdown in September and October was owing to the fact that as the dead plants disintegrated only healthier ones remained to be sampled. The decline in average root diameter in October and in the other factors in September and October indicates that some of the larger volunteer first-year plants were undoubtedly included in the samples.

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Control of the Potato Tuberworm in Potatoes[1]

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ABSTRACT

In California, the potato tuberworm, *Gnorimoschema operculella* (Zell.) is especially difficult to control in potato varieties such as Kennebec which produce tubers close to the surface of the soil. Chemical control measures are aimed at preventing population increases in foliage during the growing period so that fewer moths and larvae will be present to infest tubers when the crop matures. Insecticides tested as foliage sprays in field trials conducted during 1958 and 1959 included DDT, endrin, Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide), Guthion® (*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3-(4H)ylmethyl) phosphorodithioate), Sevin® (1-naphthyl methylcarbamate), and dimethoate. A 50% calcium arsenate dust was also tested and was found to be ineffective. In the 1958 trials, five applications of each insecticide were made at biweekly intervals. In 1959, treatments were repeated at 10-day intervals for a total of six applications.

Endrin and Guthion were the most effective materials tested. Guthion applied at the rate of 0.75 pound per acre appeared to be superior to endrin used at the rate of 0.6 pound per acre in killing larvae in their leaf mines and in controlling adults. Thiodan applied at the rate of 1.0 pound per acre gave excellent results in 1958 but control of larvae in foliage with this material in 1959 was poor. Sevin applied at 1.5 pounds per acre was about as effective as endrin applied at 0.6 pound.

Dimethoate applied at the rate of 1.0 pound per acre gave only fair control of larvae in the foliage. DDT at a dosage of 2 pounds per acre had little effect on larvae in the foliage.

Despite excellent control of larvae in the foliage with endrin and Guthion, only 51% control of larvae in the tubers of the Kennebec variety resulted. With Russett, a deeper-setting variety, endrin gave 81% control and Guthion 75%. Undoubtedly these infestations developed as a result of flights of moths from heavily infested nearby areas, indicating that although a producer may keep his field free of infestation during the growing season he may still harvest appreciably infested tubers if nearby sources of moths are disregarded.

In 1958, DDT and Guthion caused populations of the potato aphid, *Macrosiphum solanifolii* (Ashm.), to increase 6-fold and nearly 12-fold, respectively. Guthion and Sevin resulted in 7-fold and 20-fold increases in populations of the green peach aphid, *Myzus persicae* (Sulz.) in 1959.

The potato tuberworm, *Gnorimoschema operculella* (Zell.), has long been a pest of potatoes in California. Graf (1917) recorded the presence of this insect in the State as early as 1856. It occurs in the major potato-producing areas of central and southern California but does not occur in the extreme northern part of the State. During the growth of the plant, the larvae live as miners in the foliage and stems. They infest tubers in the soil prior to harvest and may also attack tubers in storage. Damage to the plants is usually of minor importance in California but on occasion infestations have been so severe that young plants have been destroyed and older vines severely injured. The insect is most damaging to potato tubers. If infestations are allowed to develop in the foliage during the season, large numbers of larvae and moths are present when the vines normally mature. Irrigation water is withheld prior to harvest to allow the soil to dry. In heavy soil many cracks appear along the tops and sides of the beds. Moths deposit eggs on the soil and the larvae hatching from these eggs enter crevices and eventually invade the tubers. Older larvae may also leave the dying foliage and enter soil openings to infest tubers. Thus at harvest time larvae of all stages may be found in the potatoes.

A number of factors influence tuberworm infestations. The most important sources of infestation are old tubers left in the ground after harvest; volunteer plants that grow from these; and piles of cull potatoes that may be left in the vicinity of fields. Tomatoes are commonly attacked but they are not as suitable a host as potato. Moths which develop in tomato fields, however, may move into potato plantings late in the season.

The depth to which the tubers are covered with soil greatly influences the degree of infestation. Lloyd (1946b) working in Australia, and Langford (1933) in Maryland, showed that the degree of tuberworm infestation of potatoes in the soil varied inversely with the soil coverage. Hofmaster (1949) obtained similar results. His data showed that tubers covered with 2 inches or more of soil were not attacked. Varieties of potato that set tubers close to the surface of the soil are more heavily infested than are deeper-setting varieties. The Kennebec, a shallow-setting variety, widely used in the production of potato chips, is one of those most susceptible.

The longer tubers remain in the soil after maturity the more subject they are to tuberworm damage. In areas where potatoes are grown for the production of potato chips, infestations are frequently high because growers and processors prefer to leave the potatoes in the ground and harvest them as needed over a prolonged period.

Essig (1926) mentions several Solanaceous weeds occurring in California as hosts of the potato tuberworm. Surveys conducted over the past 7 years have shown that weeds are of little or no importance in fostering tuberworm populations. The only weed host observed in these surveys was white horse nettle, *Solanum elaeagnifolium* Cav. This weed was not heavily infested and was attacked only if growing near infested potato fields.

Control of the potato tuberworm in potatoes is difficult to achieve in California. Chemical control measures are directed towards preventing increased populations in the foliage. This can be accomplished, but where sanitary and cultural practices are neglected, severe infestations may occur in tubers despite intensive application of insecticides.

There are many references in the literature to the effectiveness of DDT sprays and dusts: Lloyd (1946a), Hofmaster & Anderson (1948), Hofmaster (1949), Anderson & Reynolds (1950) and Dudley *et al.* (1952). DDT was widely used for controlling tuberworm in California

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for about 10 years. As early as 1954, experiments in Kern County indicated that the commonly used 5% DDT dust applied by aircraft was not providing satisfactory control. DDT sprays were more effective but, at best, they yielded only about 80% control.

Widespread failures with DDT occurred in the San Juan Valley, near Hollister, during 1957. In that same year, failures with DDT also occurred in the southern San Joaquin Valley, near Delano. Since that time DDT has given consistently poor results.

Experiments were conducted near Hollister in 1958 and 1959 to investigate the effectiveness of several other insecticides in controlling the potato tuberworm.

METHODS AND MATERIALS.—The trials for 1958 were conducted in a 15-acre field planted with the Kennebec variety. Approximately 9.5 acres of the field were divided into 20 plots, each 68 feet (24 rows) wide and 300 feet long (0.47 acre). Each treatment, including the check, was replicated four times. The insecticides used were DDT, endrin, Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6.9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide) and Guthion® (*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3-(4H)-ylmethyl) phosphorodithioate). All were formulated as commercial emulsifiable concentrates and were applied with a tractor-mounted power sprayer at 70 gallons of spray per acre. The spray boom was arranged so that three nozzles were directed over each row. The amounts of toxicant applied and the number of applications made are shown in table 1.

The remainder of the field was treated by the grower biweekly with endrin. He began by applying two sprays, the first on July 30 and the second on August 13. He then changed to a 1.5% endrin dust at 30 pounds per acre for the remainder of the season. The last treatment was applied on October 9.

Counts to determine the effect of the insecticides on larvae occurring in the foliage were made on August 19, 26 and September 9. All of the leaves on 80 plants (20 per replication) were examined for each treatment and the number of live larvae present on each plant was recorded.

The four center rows of each plot were harvested on October 16 with a two-row potato digger. Ten samples of 50 tubers were selected at random from each of the four replicates and examined for tuberworm injury. The tubers were separated into categories according to degree of infestation. Tubers with 1 to 2 larval burrows were classed as lightly infested, those with 3 to 5 burrows were designated medium, and if 6 or more burrows were found, the tubers were classified as heavily infested.

Table 1.—Results of spraying Kennebec potatoes for control of potato tuberworm larvae in the foliage. Hollister, Calif. 1958.

TREATMENT[a]		NO. OF TUBERWORM LARVAE IN FOLIAGE OF 80 PLANTS ON SAMPLING DATES INDICATED		
Insecticide[b]	Lb./A./Application	Aug. 19	Aug. 26	Sept. 9
Check	—	101	176	488
DDT	2.0	65	105	374
Endrin	0.4	35	10	0
Thiodan	1.0	37	17	2
Guthion	1.0	13	11	0

[a] Treating dates—Aug. 13, 27, Sept. 10, 27, Oct. 8.
[b] Applied as emulsion sprays with ground sprayer at 70 gallons per acre (g.p.a.)

Table 2.—Comparison of several insecticides applied to control the potato tuberworm in the foliage of Russett variety potato. Hollister, Calif. 1959.

TREATMENT[a]		NO. OF TUBERWORM LARVAE IN FOLIAGE OF 30 SHOOTS ON SAMPLING DATES INDICATED			
Insecticide[b]	Lb./A./Application	Aug. 5	Aug. 17	Aug. 31	Sept. 11
Check	—	72	191	358	646
Calcium arsenate 50% dust	35.0	110	388	—[d]	—
Thiodan[c]	0.75	19	43	31	74
Dimethoate	1.0	23	16	14	20
Sevin	1.5	27	9	11	2
Endrin	0.6	6	3	12	8
Guthion	0.75	2	1	0	0

[a] Treating dates—July 29, Aug. 7, Aug. 19, Sept. 1, Sept. 12, Sept. 29.
[b] With exception of calcium arsenate dust all materials applied as emulsion sprays with ground sprayer at 85 g.p.a.
[c] Dosage increased to 1.0 lb./acre on Aug. 19.
[d] Treatments discontinued because of ineffectiveness.

In 1959, the experiments were conducted in a field planted with the Russett variety. The insecticides used included calcium arsenate 50% dust, Thiodan, Sevin® (1-naphthyl methylcarbamate), dimethoate, endrin and Guthion. Except for the calcium arsenate dust, all insecticides were formulated as commercial emulsifiable concentrates and were applied six times with ground spray equipment at 85 gallons per acre using the amounts of toxicant indicated in table 2. The individual spray plots were 51 feet wide (18 rows) and 330 feet long (0.39 acre). All but one of the treatments were replicated three times. The calcium arsenate treatment was applied to an unreplicated block 48 rows wide and 330 feet long. Two applications of calcium arsenate dust were made. This treatment was discontinued thereafter because of poor results. The plot was then divided and endrin spray was applied to one half and Guthion spray to the other.

To determine the effect of the sprays on larvae infesting the foliage, counts were made following each of the first four applications. In the calcium arsenate treatment, counts were made following the first and second applications. Another count was made following the endrin and Guthion treatments over the calcium arsenate plot. All of the leaves on 30 shoots (10 per replication) in each treatment were examined and the data were recorded as numbers of larvae present on each shoot.

At harvest, on October 22, ten samples of 50 tubers were selected at random from the two center rows of each of the three replicates (1500 tubers from each treatment) and examined for tuberworm injury. Samples were not taken from the calcium arsenate plot because this treatment had been discontinued and other insecticides applied to the area later in the experiment. As in the 1958 experiment, the tubers were graded into categories according to the degree of infestation.

In both years, data were also obtained on the effect of the various insecticides on aphid populations. In the 1958 trials an aphid count was made following the second insecticide application by recording the number of aphids present on 100 lower leaves (25 leaves per replicate) selected at random from the center rows of each treatment. In 1959, aphid counts were made following the

Table 3.—Comparative effectiveness of Guthion and endrin in controlling a heavy infestation of potato tuberworm larvae in foliage of Russett variety potato. Hollister, Calif. 1959.

Insecticide[a] and Pounds/A.	No. of Larval Mines and Larvae in Foliage of 30 Shoots[b]		
	Mines	Larvae	
		Dead	Alive
Check	699	6	358
Endrin, 0.6	431	78	71
Guthion, 0.75	482	158	3

[a] Single application on August 19 as emulsion sprays with ground sprayer at 85 g.p.a.
[b] Counts made on August 31.

fourth application by selecting 45 lower leaves (15 leaves per replicate) at random from the center rows of each treatment. The potato aphid, *Macrosiphum solanifolii* (Ashm.) was the predominant species in 1958 and the green peach aphid, *Myzus persicae* (Sulz.), was most abundant in 1959.

Results.—From the data presented in table 1, it is apparent that in the 1958 experiments, repeated DDT applications had little effect in preventing tuberworm larvae from increasing in the foliage as the season advanced. Fewer larvae were found in the DDT-treated plots but populations consistently increased and paralleled those of the check. Endrin, Thiodan and Guthion were about equal in effectiveness and resulted in highly significant reductions of larval numbers. After the second application on August 27 these three materials had almost completely eliminated the tuberworm larvae from the foliage.

As indicated in table 2, tuberworm populations were higher in 1959. Extremely heavy infestations developed in the foliage of the check and in the calcium arsenate plots. It was apparent that the calcium arsenate dust did not reduce the infestation and perhaps it may have caused an increase. This treatment was not replicated and was located on an outside edge of the field; it is possible therefore that location of the plot may have been responsible for the increased infestation. Guthion was the most effective material tested in this trial as the first application

Table 4.—Tuberworm infestations in Kennebec tubers harvested from plots sprayed throughout the season with various insecticides. Hollister, Calif. 1958.

Treatment[a]		No. Infested Tubers in Each Category[c]					
Insecticide[b]	Lb./A./ Application	1–2 Mines	3–5 Mines	6+ Mines	Total Infested	Infested (%)	Reduction (%)
Check	—	148	123	393	664	33.2	—
DDT	2.0	136	120	311	567	28.4	14.6
Endrin	0.4	202	96	27	325	16.3	51.1
Thiodan	1.0	210	86	29	325	16.3	51.1
Guthion	1.0	203	98	23	324	16.2	51.0
Grower's[d] Endrin I	0.5	88	18	2	108	5.4	83.7
Grower's[e] Endrin II	0.5	132	38	6	175	8.8	73.5

[a] Treating dates—Aug. 13, 27, Sept. 10, 24, Oct. 8.
[b] Applied as emulsion sprays with ground sprayer at 70 g.p.a.
[c] Ten 50-tuber samples taken from each of four replicates in each treatment on October 16; sample of 2000.
[d] Samples taken 200 to 300 feet away from experimental plots.
[e] Samples taken 25 feet away from experimental area.

Table 5.—Tuberworm infestations in Russett tubers harvested from plots sprayed throughout the season with various insecticides. Hollister, Calif. 1959.

Treatment[a]		No. Infested Tubers in Each Category[c]					
Insecticide[b]	Lb./A./ Application	1–2 Mines	3–5 Mines	6+ Mines	Total Infested	Infested (%)	Reduction (%)
Check	—	27	10	17	54	3.6	—
Thiodan[d]	0.75	17	4	7	28	1.9	47
Dimethoate	1.00	10	8	11	29	1.9	47
Sevin	1.50	11	3	6	20	1.3	64
Guthion	0.60	5	5	4	14	0.93	75
Endrin	0.75	2	4	5	11	0.73	81

[a] Treating dates—July 29, Aug. 7, 19, Sept. 1, 12, 29.
[b] Applied as emulsion sprays with ground sprayer at 85 g.p.a.
[c] Ten 50-tuber samples taken from each of three replicates in each treatment on October 22; sample of 1500.
[d] Dosage increased to 1.0 lb./acre on Aug. 19.

almost completely eliminated the larvae, and repeated applications were highly effective in preventing larval development in the foliage thereafter. Thiodan, Sevin, dimethoate and endrin all resulted in significant reductions of larval populations. However, none of these insecticides were entirely effective in preventing larval development. Thiodan and dimethoate gave the poorest control. Appreciable numbers of live larvae in all stages of development were present in the foliage after applications of these materials. Endrin and Sevin were more effective and reduced numbers of larvae in the foliage to a low level but incipient infestations continued in these plots.

Data obtained from the application of endrin and Guthion to the heavily infested plot in which calcium arsenate failed are presented in table 3. Both insecticides were effective in reducing numbers of larvae. Guthion again appeared to be superior to endrin in killing larvae in their leaf mines.

While certain of the insecticides were highly effective in controlling tuberworm larvae in the foliage, control obtained in the tubers was not as satisfactory. With the Kennebec variety, used in the 1958 experiments (table 4), an average of 33.2% of the tubers were infested in the checks. As expected, the control obtained with DDT was extremely poor, only 14.6% better than the check. Despite excellent control in the foliage, endrin, Thiodan and Guthion resulted in only 51% control of larvae in the tubers. However, roughly two-thirds of the infested tubers in these treatments were classified in the lightly infested category (1 to 2 larval mines) whereas with DDT and the checks, the majority of the infested tubers were classified in the more heavily infested categories.

Endrin treatments applied by the grower gave much better control (74% to 84% reduction) than the endrin sprays in the experimental plots. It was also observed that samples taken from the part of the field treated by the grower, several hundred feet away from the experimental plots (Grower's endrin I), were less heavily infested than those taken nearer to the edge of the experimental area (Grower's endrin II).

Despite heavy infestations in the foliage during 1959, data presented in table 5 show that damage was low in the harvested tubers. The fact that the Russett variety used in this experiment forms tubers deep in the soil largely accounts for this. Only 3.6% of the tubers were infested in the checks. Fewest infested tubers were

found in the endrin and Guthion plots. Control with these insecticides was about equal, with 81% to 75% reduction in infestation, respectively. Fewer infested tubers were found in plots treated with Sevin than in those treated with Thiodan or dimethoate but it is doubtful if observed differences between these three materials are significant.

In addition to effects on tuberworm populations, the insecticides used in the experiments also affected the abundance of aphids on the potato foliage. The results of aphid counts are presented in tables 6 and 7. In 1958 and in 1959, endrin and Thiodan resulted in excellent aphid control. They appeared to control the green peach aphid and the potato aphid equally well. Fewer aphids were found in Thiodan-treated plots but it is questionable if Thiodan was significantly more effective than endrin. In 1958, both Guthion and DDT treatments resulted in higher populations of the potato aphid than in the checks. DDT caused a 6-fold increase, and there were nearly 12 times more aphids present in the Guthion treatment than in the check.

In 1959, Guthion and Sevin treatments resulted in an increase of the green peach aphid over populations in the untreated check. Sevin caused a 20-fold increase, and there were more than seven times more aphids present in the Guthion plots than in the checks.

DISCUSSION.—From the results obtained in these experiments DDT is no longer effective in controlling the potato tuberworm in potatoes in parts of California. Apparently a relatively high degree of tolerance to DDT has developed. This observation is confirmed by that of tomato growers in the Hollister area who are also experiencing difficulty in controlling this pest on tomatoes with DDT. Guthion and endrin provide excellent control of the potato tuberworm infesting potato foliage. Guthion appears to be somewhat more effective than endrin and is also highly toxic to the moths. Endrin has never effectively reduced numbers of adults. In 1958, endrin reduced larval populations in the foliage to zero. This was not achieved in the 1959 trials even though the dosage was increased from 0.4 pound to 0.6 pound per acre. Commercial applications also appeared to be less effective in 1959. Although not proved, there are indications that the potato tuberworm may be developing a tolerance to endrin.

Thiodan was highly effective in controlling tuberworm larvae in the foliage during 1958 but the results obtained in 1959 were poor. No explanation for these results is known.

Sevin was fairly toxic to the potato tuberworm, but where this material was used severe aphid and spider mite infestations developed. Plants declined more rapidly in the Sevin plots and dried prematurely. It was also interesting to observe that weed growth was retarded where Sevin was used because of extremely heavy aphid and spider mite populations.

Guthion also has the effect of increasing aphid populations. If this material is used, it should be combined with an aphicide or an alternate application of an aphicide such as endrin should be made.

Both endrin and Guthion are effective in controlling the potato tuberworm in foliage and can be relied upon to prevent populations from increasing within the field. It is obvious, however, that these materials cannot adequately control heavy migrations of moths that move into a field late in the season from outside sources. The probable reason for poor control of worms in tubers with these materials used experimentally was because the treated plots were located close to heavily infested checks and the less effective treatments. Large numbers of moths developed in these nearby plots, migrated, and deposited eggs on the soil or in crevices thus infesting the tubers in the better treatments. These observations suggest that although a producer may keep his field free of infestation during the growing season, he may harvest appreciably infested tubers if nearby sources of moths are disregarded in his program of sanitation.

Table 6.—The effect on populations of the potato aphid in plots treated with sprays to control the potato tuberworm. Hollister, Calif. 1958.

INSECTICIDE[a]	DOSAGE (LB./A.)	TOTAL APHIDS PER 100 LEAVES[b]
Check	—	442
Guthion	1.0	5,146
DDT	2.0	2,612
Endrin	0.4	26
Thiodan	1.0	0

[a] Treatments applied Aug. 12, 27, Sept. 10.
[b] Counts made Sept. 9; 25 leaves examined per replicate.

Table 7.—The effect on populations of the green peach aphid in plots treated with sprays to control the potato tuberworm. Hollister, Calif. 1959.

INSECTICIDE[a]	DOSAGE (LB./A.)	TOTAL APHIDS PER 45 LEAVES[b]
Check	—	284
Sevin	1.50	5,581
Guthion	0.75	2,115
Endrin	0.60	5
Dimethoate	1.00	3
Thiodan[c]	0.75	2

[a] Treatments applied July 29, Aug. 7, 19, Sept. 1.
[b] Counts made Sept. 1; 15 leaves examined per replicate.
[c] Dosage increased to 1.0 lb./acre on Aug. 19.

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Selective Insecticides for Vegetable Leaf-Miner Control and Parasite Survival[1]

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Abstract

A number of organophosphorus insecticides were tested on cantaloupes and peppers for control of a serpentine-leaf-miner (*Liriomyza munda* Frick) and survival of a parasite (*Derostenus variipes*, Cwfd.) to find insecticides with selective properties for use in an integrated control program. Ethion, Delnav® (2,3-*p*-dioxanedithiol *S,S*-bis (*O,O*-diethyl phosphorodithioate 68%–70% and related materials)) and dimethoate gave excellent control of the leaf miner with residual toxicity lasting for 9 days or more on treated foliage. Parathion and Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate) were also effective, but lacked the residual control of the other insecticides. The highest parasite survival was obtained with Delnav, Dylox® (dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate), Dibrom® (1,2-dibromo-2,2-dichloroethyl dimethyl phosphate), EPN, dimethoate, demeton and Am. Cyanamid 12008 (*O,O*-diethyl *S*-(isopropylthio)methyl phosphorodithioate). Parathion, ethion and Diazinon, some of the more effective leaf miner control agents, were highly toxic to *D. variipes*.

Delnav and dimethoate showed the greatest amount of selectivity for controlling *L. munda* and allowing survival of *D. variipes*. Both insecticides gave lasting residual control of the leaf miner and at the same time allowed 45% to 100% survival of the parasite larvae and pupae within the leaf. Insecticides with this degree of selectivity are necessary for the development of an integrated chemical-biological control program of vegetable leaf miners.

A serpentine leaf miner, *Liriomyza munda* (Frick),[2] is a major pest of cantaloupes and peppers in the Lower Rio Grande Valley of Texas. This insect problem has been augmented by the utilization of hydrocarbon insecticides. These chemicals are used for control of other insects, but unless an effective insecticide for leaf miner is used in combination with the hydrocarbon insecticide, dipterous miners build up in large numbers. Natural control from hymenopterous parasites often is an important factor in maintaining low leaf-miner populations, but the indiscriminate use of certain insecticides seriously affects the leaf miner-parasite relationship (Hills & Taylor 1951, Wene 1955, Oatman 1959).

Chlordane, lindane, aldrin, dieldrin and toxaphene were once used for leaf-miner control, but in a comparatively short time these chemicals became ineffective as control measures (Jefferson & Eads 1952, Wene 1955). Organophosphorus insecticides are now used for control of leaf miners on vegetables. Parathion is still one of the most effective chemicals after 8 to 10 years of usage in many areas. However, leaf-miner tolerance for parathion has been reported (Wolfenbarger 1958). Parathion has been widely used for miner in chemical control schedules in the Rio Grande Valley with no indications of parathion-tolerant leaf miners.

In the absence of chemical control, natural enemies sometimes help to maintain the leaf miner below economic levels. However, insecticides are required when leaf miner populations increase rapidly. The effective toxicants commonly used for control of leaf miners in the Rio Grande Valley are also detrimental to the hymenopterous parasites. Once such a chemical is applied, any balance that has existed between parasite and host is disrupted and a continual insecticide control program usually becomes necessary. The use of an insecticide selective for leaf-miner mortality and parasite survival would provide an integrated program of chemical and biological control. During 1958 and 1959 a number of organophosphorus insecticides were tested on cantaloupes and peppers in an attempt to find an insecticide that would control leaf miners and permit parasite survival. The present paper reports the results of these studies.

Methods and Results.—Insecticides were applied with hand sprayers to plants in small plots bordered by untreated rows. Tests were replicated four times and arranged in randomized complete blocks. All toxicant formulations consisted of emulsifiable concentrates except for EPN and Dylox® (dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate) which were formulated as a wettable powder and a soluble powder, respectively. Leaf-miner larvae in leaf samples of Rio Gold cantaloupe and Yolo Wonder pepper were counted 2, 5 and 9 days after treatment in most tests. In the experiments with peppers, leaflets were selected at random. Cantaloupe samples consisted of leaves selected from a particular location on each plant.

A primary objective was to determine the effect of insecticides upon hymenopterous parasites of *L. munda*. Although several parasites aid in natural control of leaf miners in this area, the results of these tests only reflect the toxicity of the insecticides to larvae and pupae of *Derostenus variipes* (Cwfd.), the most important parasite of *L. munda* during 1958 and 1959, locally. Unlike most other hymenopterous parasites of leaf miners in the Rio Grande Valley, *D. variipes* kills its host before the miner emerges from the leaf mine tunnel and thus, the parasite pupates within the latter. Parasite survival was estimated by holding the 2-day leaf samples in rearing cages at 25° C. until adult parasite emergence was completed. The adults were collected, identified to species and counted.

Test No. 1.—During the summer of 1958, nine insecticides were tested on cantaloupes for leaf-miner control and parasite survival. Plots consisting of 3 rows, each 25 ft. long, were treated August 9 with a 3-gallon pressure sprayer at a rate of 10 gallons per acre when the plants had 8 to 12 true leaves. The 2-, 5- and 9-day counts were based on 10, 6 and 3 leaf samples, respectively. Leaves from each sample were observed through a binocular microscope for living leaf miner larvae.

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[2] Species determination by R. H. Foote, Entomology Research Division, U. S. Department of Agriculture. *Derostenus variipes* identified by B. D. Burks, of the same Division.

All of the insecticides included in this test provided initial control (2-day counts) of *L. munda* (table 1). The materials with the grestest residual toxicity were determined by the 5-day counts. At this time, ethion and Delnav® (2,3-*p*-dioxanedithiol *S,S*-bis(*O,O*-diethyl phosphorodithioate 68%–70% and related materials) were far superior to the other treatments. Parathion and Trithion® (*S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate) provided some protection against the miners five days after treatment. The remaining chemicals were ineffective as residual control agents. The Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate) and Metacide (a formulation of methyl parathion and parathion) emulsifiables were more than 2 years old and may have partially deteriorated. Regardless of the insecticides used, 9 days after plant treatment, unsprayed new growth showed no reduction in leaf miner attack. Additional samples of older treated leaves were taken from the ethion, Delnav and parathion plots 9 days after spraying; at that time, leaves previously sprayed with ethion and Delnav had fewer leaf miners than the untreated controls.

Insecticide treatments influenced survival of the hymenopterous parasite, *D. variipes*. Am. Cyanamid 12008 (*O,O*-diethyl *S*-(isopropylthio)methyl phosphorodithioate), demeton, Trithion and Delnav were the least toxic to *D. variipes* larvae and pupae. Guthion® (*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3-(4*H*)-ylmethyl) phosphorodithioate), ethion, parathion and Diazinon greatly reduced parasite populations. A high standard of deviation in the parasite count was caused by localized areas of heavily or sparsely parasitized leaf miners within the experimental block.

Test No. 2.—To determine the effect rates of application of insecticides on miner control and parasite survival, the best materials in the previous experiment were applied to cantaloupe at various amounts per acre. Plots, each containing one 75 ft. row, were sprayed on August 19 at a rate of 18 gallons per acre when the plants had 5 to 6 true leaves. Leaf miner counts, 2 and 5 days after application were recorded from 8 and 6 leaves, respectively.

All of the materials at the rates tested gave significant leaf-miner control initially, based on the 2-day samples (table 2). After 5 days, ethion at 0.25 or 0.5 lbs., Delnav at 0.5 lbs. and parathion at 0.25 or 0.5 lbs. per acre, provided the best residual control. The population of adult leaf miner in the field disappeared after the sprays were applied, thus the 5-day counts indicated better control from the insecticides than in the first test.

Table 1.—Control of *Liriomyza munda* with insecticides and survival of the parasite, *Derostenus variipes*, on Rio Gold cantaloupes. Weslaco, Texas. 1958.

Treatment	Actual Toxicant (lb./a.)	Leaf Miners per Leaf After Days Indicated			Emerged Parasites per Leaf[a]
		2	5	9	
Demeton	0.3	3.2	55.7	66.5	4.4
Ethion	0.6	0.0	1.0	78.8	1.7
Delnav	0.6	3.5	1.7	70.7	4.3
Am. Cyanamid 12008	0.5	0.5	52.7	76.5	4.8
Guthion	0.2	3.2	52.7	75.0	0.6
Parathion	0.2	0.0	25.6	79.5	2.8
Trithion	0.2	3.8	46.4	77.5	4.4
Diazinon	0.6	0.0	53.5	73.8	3.0
Metacide	0.6	0.0	61.0	84.8	3.9
Check		38.7	74.9	63.2	7.8
L.S.D. @ 5% level		3.9	13.4	n.s.	2.8
@ 1% level		5.3	18.1	n.s.	3.7

[a] Numbers of adult *Derostenus variipes* that emerged from 2-day leaf samples.

Table 2.—Comparison of dosages of insecticide for control of *Liriomyza munda* and survival of *Derostenus variipes* on Rio Gold Cantaloupe. Weslaco, Texas. 1958.

Treatment	Actual Toxicant (lb./a.)	Live Miners per Leaf After Days Indicated		Emerged Parasites per Leaf[a]
		2	5	
Ethion	0.5	0.0	0.3	1.6
Ethion	0.25	0.1	1.8	3.4
Ethion	0.125	0.8	11.7	5.3
Delnav	0.5	2.7	2.8	7.6
Delnav	0.25	5.6	9.3	9.1
Parathion	0.5	0.0	1.7	—
Parathion	0.25	0.3	5.0	—
Trithion	0.5	0.0	14.9	1.6
Trithion	0.25	0.4	23.5	3.3
Check		38.8	42.5	9.1
L.S.D. @ 5% level		2.8	4.8	4.1
@ 1% level		3.8	6.6	5.6

[a] Numbers of adult *Derostenus variipes* that emerged from 2-day leaf samples.

Parasite survival was affected by the chemicals and their concentration. Delnav was the least toxic to larvae and pupae of *D. variipes*. Since the parasites from the parathion-treated leaf samples were lost in rearing, no data from these samples are available.

Test No. 3.—Twelve insecticides were tested for leaf-miner control on peppers in December 1958. Plots consisting of two rows 50 ft. long, each bordered by a non-treated row, were sprayed with a 3-gallon knapsack sprayer at a rate of 50 gallons per acre on December 11. An appropriate wetting agent (Du Pont Spreader-sticker) was used to aid in obtaining uniform coverage. At the time of application, fruit was maturing on the plants. Samples were collected 2 days after treatment by randomly selecting 15 leaflets from each plot for leaf-miner counts. Initial data were taken by recording the number of live miners visible within the mines when viewed through the binocular microscope. Because this method of obtaining data is time consuming and tedious, another counting procedure was tried. The examined leaf samples were held in pint ice-cream cartons for 6 days until the larvae had emerged and pupated. Counts of pupae were made and compared with the live miner counts obtained previously.

Poor control of *L. munda* was obtained with most of the insecticides applied to infested peppers (table 3). Dylox, Diazinon and Dibrom® (1,2-dibromo-2,2-dichloroethyl dimethyl phosphate) gave satisfactory control as indicated by counts of both the live miners and the pupae. The counts of larvae and pupae compared favorably; therefore, all further insecticide tests were evaluated by the pupal-count method. Reliable parasite-survival data were not obtained because of low insect numbers.

Test Nos. 4 and 5.—In the summer of 1959, two experiments were conducted on cantaloupes and peppers to compare the effect of the host plant upon insect mortality by insecticides and to screen toxicants suitable for leaf-miner control and parasite survival. One experiment in-

Table 3.—Control of *Liriomyza munda* with insecticides on Yolo Wonder peppers 2 days after treatment. Weslaco, Texas. 1958.

Treatment	Actual Toxicant (lb./a.)	Leaf Miners per Leaf	Miner Pupae per Leaf
Ethion	0.50	3.7	4.2
Phosphamidon	0.50	2.3	2.1
Parathion	0.50	2.1	2.2
Parathion	0.25	2.6	3.4
Malathion	1.00	2.4	4.9
Phosdrin	0.25	5.6	8.9
Dylox	1.00	0.4	0.7
Methyl parathion	0.50	1.6	3.1
Delnav	0.50	1.8	5.6
Demeton	0.25	4.3	8.6
Diazinon	0.50	0.7	0.5
Guthion	0.50	1.7	1.5
Dibrom	1.00	0.4	0.9
Check		8.7	9.5
L.S.D. @ 5% level		1.7	2.0
@ 1% level		2.3	2.6

cluded seven organophosphorus toxicants, usually considered as contact sprays, along with DDT and heptachlor. Five systemic insecticides and parathion were used in the second test on each crop. The procedures utilized for all tests were similar. Each plot consisted of one row 50 ft. long and was bordered by an untreated row. Spray applications were made with a carbon-dioxide-pressured small plot sprayer at a rate of 10 gallons per acre to peppers on August 13 and to cantaloupes on August 15. The pepper plants had been defoliated previously and all leaves were relatively young. Cantaloupe plants contained 3 to 5 leaves at the time of application. Leaf-miner control was determined by counts of pupae.

All of the organophosphorus contact insecticides gave significant control of the leaf miner in cantaloupe and pepper leaves initially (table 4). DDT and heptachlor were ineffective throughout the test on the two crops. Five days after treatment ethion and Delnav on both vegetables and Diazinon on peppers gave the most effective control. Nine days after application, ethion and Delnav still provided some residual toxicity. Delnav did not produce an extremely high initial kill even though it had great residual toxicity.

Parasite survival from insecticides varied greatly on the two crops; a higher percentage of survival occurred in treated peppers than in treated cantaloupe leaves. Delnav, Dylox, Dibrom and EPN were among those phosphates least toxic to *D. variipes* while parathion and ethion were extremely toxic. DDT and heptachlor were not as harmful to larvae and pupae of *D. variipes* as might be expected.

Dimethoate proved to be an excellent, persistant insecticide for leaf-miner control on both cantaloupes and peppers (table 5). Nine days after treatment it gave control comparable to that obtained with ethion and Delnav in the previous test. Initial control was also obtained with phorate and Am. Cyanamid 12008 on both vegetables.

As with the contact poisons, greater parasite survival occurred in peppers treated with systemic insecticides. For the most part the systemics were much less toxic to *D. variipes* than parathion. Demeton, Phosdrin® (1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate) and dimethoate did not affect survival of the parasites when applied to peppers.

Discussion.—*Liriomyza munda* was responsible for most of the leaf miner damage to vegetables in this area during 1958 and 1959. *L. pusilla* and *L. propepusilla* (*subpusilla*) have been recorded as severe pests previously (Mayeaux & Wene 1951, Wene 1953). Recently the "*pusilla*" complex was separated into a number of species which includes *L. munda* (Frick 1956, 1957) and it is entirely possible that this species was formerly identified as either *L. pusilla* or *L. propepusilla*.

The data obtained in these experiments give an indication of larvicidal and possibly ovicidal residual toxicity rather than adult mortality because of the techniques employed. The small plot size and the utilization of border rows provided an ample number of flies for continual oviposition throughout the test period. Control of the larvae is dependent upon leaf penetration by the insecticide. With few exceptions better leaf-miner control and lower parasite survival was obtained on cantaloupe than on peppers. Diazinon was more effective on peppers than on cantaloupes. The control of adult flies is not

Table 4.—Control of *Liriomyza munda* with insecticides and survival of *Derostenus variipes* on cantaloupes and peppers. Weslaco, Texas, 1959.

Treatment	Actual Toxicant (lb./a.)	Leaf Miners per Leaf After Days Indicated on						Emerged Parasites[a] per Leaf from	
		Cantaloupe			Pepper				
		2	5	9	2	5	9	Cantaloupe	Pepper
EPN	0.25	0.3	6.0	17.1	0.1	1.1	1.0	1.4	0.7
Parathion	0.25	0.1	4.0	19.5	0.1	1.3	1.1	0.2	0.3
Delnav	0.50	1.6	0.7	3.4	0.3	0.4	0.2	2.3	0.7
Ethion	0.50	0.5	0.2	7.7	0.1	0.5	0.5	0.8	0.5
Dibrom	0.50	6.9	11.1	—	0.4	1.9	1.1	1.0	0.9
Diazinon	0.25	0.1	13.7	—	0.0	0.4	0.8	0.6	0.5
Dylox	0.50	0.1	9.3	17.6	0.4	1.9	—	1.7	0.7
DDT	1.00	18.1	6.4	—	1.5	1.8	1.3	2.0	0.5
Heptachlor	0.25	15.3	9.2	—	1.2	1.8	—	1.7	0.6
Check		22.8	14.3	18.8	1.1	1.7	0.9	5.3	0.8
L.S.D. @ 5% level		5.0	5.6	—	0.3	0.8	—	1.2	0.2
@ 1% level		6.8	7.5	—	0.4	1.0	—	1.7	0.3

[a] Numbers of adult *Derostenus variipes* that emerged from 2-day leaf samples.

Table 5.—**Control of *Liriomyza munda* with systemic insecticides and survival of *Derostenus variipes* on cantaloupes and Peppers. Weslaco, Texas. 1959.**

Treatment	Actual Toxicant (lb./a.)	Leaf Miners per Leaf After Days Indicated on						Emerged Parasites[a] per Leaf from	
		Cantaloupe			Pepper				
		2	5	9	2	5	9	Cantaloupe	Pepper
Demeton	0.25	2.5	2.9	9.4	0.6	1.7	0.9	2.1	1.1
Phorate	0.50	0.1	2.4	11.1	0.1	0.7	0.9	1.4	0.5
Phosdrin	0.25	4.4	4.5	—	1.2	2.7	—	1.8	1.2
Dimethoate	0.50	0.0	0.1	3.1	0.0	0.1	0.3	1.3	1.2
Am. Cyanamid 12008	0.50	0.1	2.6	7.6	0.2	1.1	1.0	1.2	0.8
Parathion	0.25	0.0	1.0	12.5	0.2	1.4	1.2	0.2	0.5
Check		13.0	3.8	8.2	1.7	2.1	0.9	7.2	1.0
L.S.D. @ 5% level		3.2	2.8	—	0.3	0.7	—	1.5	0.3
@ 1% level		4.4	3.8	—	0.4	1.1	—	2.0	0.5

[a] Numbers of adult *Derostenus variipes* that emerged from 2-day leaf samples.

considered important with the commonly used leaf-miner insecticides because of continual adult emergence from pupae in the soil. The possibility that certain chemicals affect oviposition by the adults through repellency or other means is now being investigated.

The biology of a parasite species is important when chemical effects upon the population are considered. *D. variipes* pupates in the miner tunnel of the leaf. Most of the other major parasites of leaf miners in this area do not kill the host and do not pupate until the miner has emerged from the tunnel and entered the soil. Parasites such as *D. variipes* are subjected to lethal chemical applications in all developmental stages while parasites that allow the host to enter the soil are protected from chemicals during the pupal stage.

Insecticides varied in toxicity to *L. munda* and *D. variipes*. Ethion, Delnav and dimethoate were excellent toxicants for leaf-miner control with long residual toxicity. In some instances, especially on cantaloupes, new growth became infested while the older treated leaves were protected against severe miner damage for 9 days. Parathion and Diazinon usually gave good initial control and some residual toxicity. Most of the other phosphates tested provided initial control, but no residual action. Delnav, Dylox, Dibrom, EPN and the systemic insecticides, excluding phorate, were the least toxic to larvae and pupae of *D. variipes*. As with the leaf miner, greater parasite mortality occurred with treated cantaloupe than with treated peppers. Parathion, ethion and Diazinon, some of the more effective for leaf-miner control, and Guthion were highly detrimental to the parasite.

DDT and heptachlor had little effect upon the leaf miner. The parasite population was reduced by the hydrocarbons, but certain phosphate insecticides caused more parasite mortality than did either DDT or heptachlor. The phosphates had the advantage in that they also caused significant leaf-miner mortality. It is possible that the hydrocarbons, because of their persistence, are extremely detrimental to the adult parasite populations.

Many of the criteria required for an integrated control program as described by Smith & Hagen (1959) exist here. The economic threshold of the pest is rather high, natural enemies are indigenous and capable of holding the leaf miner in check, and selective insecticides are available when biological control fails. One disadvantage is that nonselective insecticides may be required for the control of pests other than the leaf miner on some crops, thus, adversely affecting natural control.

Delnav appears very promising as a chemical available for use in an integrated control program. It will give excellent residual toxicity for the leaf miner and permit parasite survival. Dimethoate also gives indication of being a highly selective insecticide. Additional chemicals are available for good leaf-miner control, but they are also very toxic to *D. variipes*. Insecticides with selective toxicity are necessary for the development of an integrated chemical-biological control program of vegetable leaf miners.

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Light Intensity and the Attraction of Mosquitoes to Light Traps[1]

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Abstract

A method is described for evaluating the performance of light traps. The mosquito collections of light traps operated within a few feet of each other showed important differences, especially when the mosquitoes were present in large numbers. These differences were eliminated when the traps constantly changed position. There was no evidence that moving traps caught fewer mosquitoes than stationary traps.

Lighted traps caught more mosquitoes than unlighted traps although the latter caught substantial numbers of *Culex* but not *Aedes* mosquitoes.

The light intensity of the bulb illuminating a trap was directly related to the numbers of individuals of a species taken by that trap, except in the case of *C. p. quinquefasciatus* females. In fact the number of mosquitoes appeared to be directly proportional to the light intensity, with the exception noted. No repellency was noted at the highest intensities tested.

Results with *C. p. quinquefasciatus* females were not yet clear-cut but females of this species appear to show a low attractance to light on dark nights but not on light ones. The effect of moonlight on females of this species is so pronounced that unlighted traps probably give a much better indication of abundance than lighted ones.

Light traps have proved to be useful in the study of mosquitoes both for conducting surveys and for monitoring populations. They are potentially useful for behavioral studies as well, if properly used. The problem of evaluating light trap performance is one that has not been solved, however. In this kind of work there are two outstanding sources of variation, that which is due to changes in number of active mosquitoes from one night to the next (nightly variation), and that which is caused by the location of the trap (position variation). The first problem can be solved by operating several traps on a given night but this introduces the second large source of variation, that caused by position. The second problem can be solved by operating a trap in the same place each night but this in turn introduces nightly variation. In practice, most evaluation studies have involved operating two or more traps in the same places over a period of time with the factors to be evaluated being alternated between (or among) the traps (see, for example, Reed 1959). Techniques for evaluating data of this type, however, are extremely inefficient so that large numbers of tests must be carried out. This introduces still new sources of error such as seasonal variability in behavior. Results from such experiments are generally inconclusive unless the factors tested have extremely large effects.

A rotary device was described by Bargren & Nibley (1956) for evaluating the effect of wave length of light emitted on capture of mosquitoes by light traps. The device consisted basically of a stand from which four traps were suspended. The stand rotated very slowly so that the traps changed places continually. The experimental design was very efficient in that variability owing to position could be minimized or possibly even eliminated. Unfortunately, there was no statistical analysis of the results to determine whether such variability had been eliminated. A somewhat similar device was described by Edgar & Herndon (1957) which should work less well since all choices are not visible simultaneously to the insect.

The objective of the present study was to develop a device for evaluating the effect of factors influencing the operation of light traps.

Materials and Methods.—The basic device used, a rotary stand from which four light traps were suspended, was one of those described by Dow *et al.* (1957).[2] The chief modification of the device was to make the center post stationary so that only the arms revolved; in the original model the entire stand was attached to a base which rotated. The original device revolved unevenly when there was wind but the modified device turned very evenly. The light traps were suspended at the ends of the arms, 1.5 m. from the center post. Adjacent traps were therefore separated by about 2 m. and opposite traps by about 3 m. The lights in the traps were approximately 1.5 m. from the ground. It required approximately 4 min. for a complete revolution of the device so the traps travelled approximately 2.4 m./min.

The traps utilized for the study were "American model," New Jersey light traps as described by Mulhern (1953) except that the traps were unscreened. The four traps used were as comparable as possible. At the beginning of the study the air flows of the traps were 90 to 98 linear m./min. as measured by the method of Loomis (1959). At the end of the study the air flows were 81 to 98 m./min.

The light bulbs used were of the vibration service type currently in use in mosquito abatement agencies in California and were inside frosted, white bulbs of varying wattages.

The locality in which the study was conducted was a pasture near Riverdale, Fresno County, California. The device was placed in a fairly exposed position. It was provided with an automatic timer so that all traps operated and the device turned from 8 p.m. to 6 a.m. during the summer and from 6 p.m. to 6 a.m. after the fall equinox. The traps were checked and serviced after each night of operation. All tests were made from June 24 to October 22, 1959. The species dealt with in this study are *Aedes nigromaculis* (Ludlow), *Culex pipiens quinquefasciatus* Say, *C. tarsalis* Coquillett, and *C. peus* Speiser.

Results.—*Effect of Rotary Device in Reducing Variability Owing to Position.*—A series of tests was conducted to determine (a) whether the revolving device reduced variation as compared with the same device when not

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revolving, and (b) whether the four traps caught similar numbers of mosquitoes. For these tests each trap was provided with a 25-watt bulb. The tests were done from June 24 to August 5. The hypothesis that the mosquitoes taken in a given night were equally distributed among the four traps was tested by chi square. On 8 of 9 nights when the rotary device was stationary, the chi square values were significant ($p < .05$). In contrast, there were no significant chi squares during 13 nights when the device was rotating. In addition, the total chi square for the 13 nights was not significant. These results indicate (a) that the rotary device decreases the amount of variability between the four traps, and (b) that no differences were shown in the attractancy or collecting power of the four traps. The mosquitoes taken during these tests were 51% *A. nigromaculis*, 31% *C. tarsalis*, 14% *C. p. quinquefasciatus* and 4% others.

The traps were run for an additional 6 nights (August 24 to September 1) during which the bulbs were unscrewed slightly so that they did not light; the fans did operate, however. These darkened traps caught substantial numbers of mosquitoes and the chi square was significant on 1 of the 6 nights. These results indicate that the collecting power of the four traps was similar when the traps were not illuminated as well as when they were lighted. In these six tests the unlighted traps caught 51% *C. p. quinquefasciatus*, 45% *C. tarsalis* and 4% *C. peus*.

It is important to know not only that rotation decreases variation among the traps tested on a given night but also to what extent it decreases such variation. This relationship is shown in figure 1 where the mean number of mosquitoes per trap is plotted against the standard deviation of the mean[3] for each of the nights when the device was or was not rotating. (These data are from the first series of experiments involving lighted traps only.) The standard deviation is used because it is a measure of the dispersion of a frequency distribution although it has little meaning in an absolute sense when based on only four values as in the present case. The graph demonstrates that the device reduces variability tremendously at high population densities, and very little at low densities. The one night when the traps were not rotating and the mosquitoes appeared to be equally distributed among the four traps (*i.e.*, the chi square was not significant) was the night that the lowest numbers of mosquitoes (7.8/trap) were taken in the four stationary traps. It will be noted that this point falls on the line for the rotating traps. These data indicate that the rotary device was useful in reducing variability among the traps when the average per trap was about 10 mosquitoes; at lower densities it seemed to be of no value. It will also be noticed from the graph that, although strict comparisons cannot be made, the average level of catch was not outstandingly different in the two kinds of tests; that is to say that rotation does not appear to lower appreciably the number of mosquitoes caught.

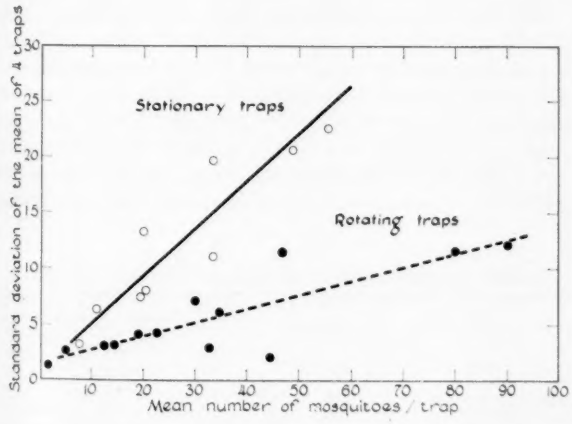

FIG. 1.—Standard deviation of the mean plotted against the mean number of mosquitoes taken by rotating and stationary traps.

Table 1.—Protocol for placing lights (+) in traps to test the collecting power of lighted and unlighted traps and the effect of pattern of rotation.

	NIGHT							
TRAP	1	2	3	4	5	6	7	8
1	−	−	+	+	+	+	−	−
2	+	+	−	−	+	−	−	+
3	+	−	−	+	−	+	+	−
4	−	+	+	−	−	−	+	+

The Effect of Pattern of Rotating Lights on Catch of Trap.—It was desired to provide the four traps with lights of different intensities to study the effect of this factor. Thus the question arose whether an unlighted trap would catch as many mosquitoes when it was following another unlighted trap as when it was following a lighted trap; that is, does the pattern of rotating lights affect the collecting power of any one of the traps? This question was approached by using a block design, covering 8 nights. A typical protocol is shown in table 1. On each of the 8 nights two of the traps were unlighted and two were lighted. On 4 of the 8 nights the two lighted traps were adjacent to each other and on the other 4 they were opposite each other; the opposite and adjacent designs were alternated from 1 night to the next. The four adjacent arrangements were assigned randomly to their 4 nights but in such a way that each arrangement was tested only once. Since there are only two opposite arrangements, each was tested twice. A design of this type was tested on three occasions (August 10 to September 27) so that 24 nights of trapping were included. The mosquitoes taken on these nights are shown in table 2.

Significantly more mosquitoes of each sex of each species were taken by the lighted than by the unlighted traps except in the case of female *C. p. quinquefasicatus* which were equally distributed between the two kinds of traps. The figures for female *C. p. quinquefasciatus* were examined further on a nightly basis. Of the 24 nights in question there were 3 nights in which the two lighted traps caught significantly ($P < .05$ by chi square) more females than the two unlighted traps and there was 1 night when the reverse was the case. In one of the three experiments (of 8 nights each) the difference between numbers of females caught in the lighted and unlighted traps was significant (chi square = 4.63, $.05 > P > .01$) but this disappeared when the three experiments were pooled.

[3] Standard deviation of the frequency distribution.

Table 2.—Numbers of mosquitoes taken in lighted and unlighted traps in 24 nights of trapping.

Species	Males			Females			Total		
	Lighted	Unlighted	P[a]	Lighted	Unlighted	P	Lighted	Unlighted	P
A. nigromaculis	126	0	<.001	882	7	<.001	1,008	7	<.001
C. tarsalis	13	4	<.05	485	117	<.001	498	121	<.001
C. p. quinquefasciatus	127	22	<.001	220	191	>.2	347	213	<.001
C. peus	6	0	<.05	61	20	<.001	67	20	<.001
Total	272	26	<.001	1,648	335	<.001	1,920	361	<.001

[a] Probability that difference between figures for lighted and unlighted traps could be due to chance alone.

Close comparison of the numbers of mosquitoes taken by traps in the opposite and adjacent arrangements cannot be made because these experiments were necessarily conducted on different nights. Comparisons were made, however, by calculating the mean difference in number of mosquitoes collected by traps in the opposite and adjacent positions. Some summaries of these data are shown in table 3.

It may be seen, for example, that *A. nigromaculis* collections in the two lighted traps differed by an average of 3.17 mosquitoes when the traps were opposite each other and by 5.00 when the traps were adjacent to each other; there is enough variability in the data so that an average difference this large or larger would be expected to occur about once in three trials by chance alone.

In summary, although close analysis of these data is not possible because of the poor experimental design, there was no evidence to indicate that the pattern of rotating lights affected the catch of a given trap; a trap appeared to catch as many mosquitoes when following an unlighted trap as when following a lighted one. For this reason in the experiments which follow, in which four different conditions were tested on the same night, a single pattern of rotating lights was used in all tests.

The Effect of Light Intensity on Numbers of Mosquitoes Taken.—In these experiments the four traps were equipped with 25-, 50-, or 75-watt incandescent bulbs; one trap was provided with a burned-out 25-watt bulb. The order of the wattages in the rotating traps was 0-, 75-, 25-, and 50-watts, respectively. Each bulb was shifted to the trap next ahead of it nightly so that an experiment involved four nights with each bulb being used once in each trap. Measurements of light intensity of the bulbs were made with a Weston (model 614) foot-candle meter which was placed along the edge of the rim of each trap about 18 cm. from the bulb. A number of measurements were taken of each bulb and the median reading used. These medians were about 42, 110, and 150 foot-candles, respectively, for the 25-, 50- and 75-watt bulbs. A latin square analysis of variance was used to analyze the data. This analysis allowed the sorting out of three factors, nightly variation, variation owing to trap, and variation owing to light intensity. The experiment was done four times from September 28 to October 22 and the data pooled. The results of this analysis are shown in table 4.

The analysis was done on total numbers of individuals of a species except in the case of *C. p. quinquefasciatus* where evidence has already been presented to show that males and females act dissimilarly with respect to response to light. The variation among traps was insignificant in all cases. The variation in numbers of mosquitoes taken from night to night was significant in three out of the four cases and highly significant in the cases of *A. nigromaculis* and females of *C. p. quinquefasciatus*. The variation owing to light intensity was also significant in three out of four cases and highly significant in two of the three.

The numbers of adults of each sex taken during the 16 nights of trapping as plotted against the light intensity in foot-candles is shown in figure 2. The number of mosquitoes collected was directly related to the light intensity in all cases; it will be remembered, however, that this relationship was not significant in the case of *C. p. quinquefasciatus* females. The linearity of the points indicates that the number of mosquitoes taken was directly proportional to the light intensity tested.

The total number of female *C. p. quinquefasciatus* taken in each of the four experiments is shown in table 5. These females appeared to be attracted to light in experiments 2 and 4 (and possibly 1) and to be repelled by light in experiment 3. These data and all others on *C. p. quinquefasciatus* females were analyzed in light of the temperature and relative humidity on the night of the experiment with no obvious correlation being found. They were also

Table 3.—Mean difference in numbers of mosquitoes taken by two traps when adjacent or when opposite each other on the four-trap rotary device.

Lighting Condition	Species	Opposite Traps	Adjacent Traps	P[a]
Lighted	*A. nigromaculis*	3.17	5.00	0.7
	C. tarsalis	5.00	4.43	.8
	C. p. quinquefasciatus ♀♀	2.00	2.29	.9
	All species	5.33	6.14	.8
Unlighted	*C. p. quinquefasciatus* ♀♀	2.50	1.79	.7
	All species	4.17	2.50	.6

[a] Probability that difference could be due to chance alone.

Table 4.—Analysis of variance of tests on light intensity.

Species	Trap Variation			Nightly Variation		Light Intensity	
	N	F	P[a]	F	P	F	P
Aedes nigromaculis	287	1.40	>.05	4.46	<.01	23.86	<.01
Culex tarsalis	102	0.55	>.05	1.53	>.05	8.20	<.01
C. p. quinquefasciatus ♂♂	336	1.26	>.05	3.93	<.05	2.98	<.05
C. p. quinquefasciatus ♀♀	495	2.13	>.05	4.64	<.01	0.35	>.05

[a] Probability that this or larger amount of variation could be due to chance alone.

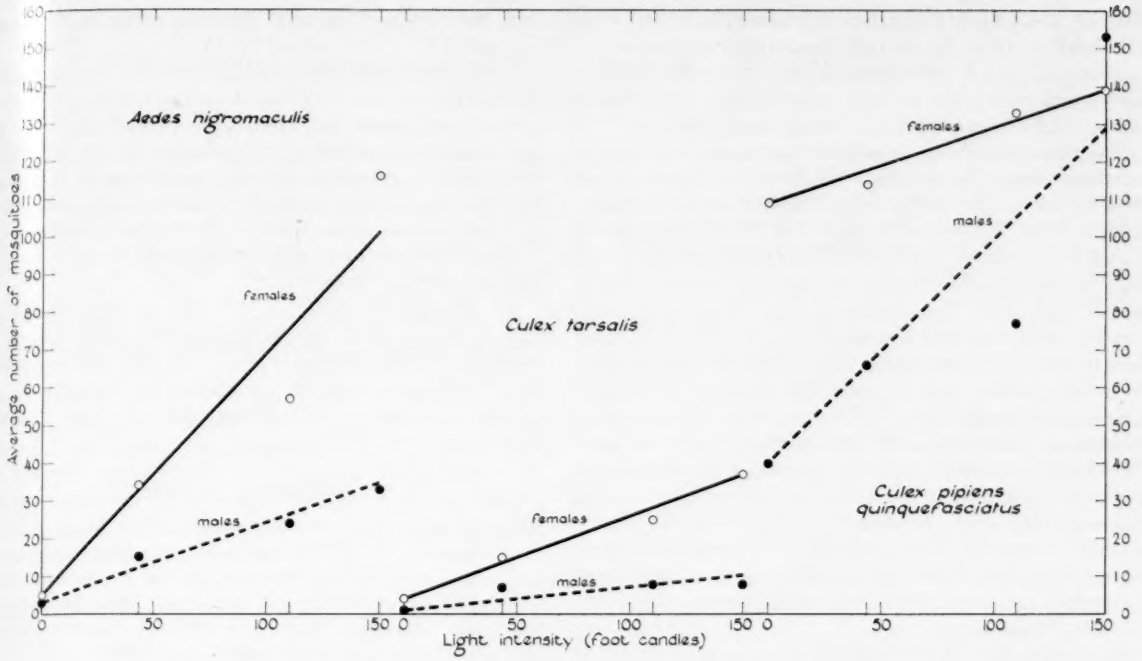

FIG. 2.—Numbers of mosquitoes taken in light traps as related to the emission of the bulb (in foot-candles).

compared with data on lunar cycles which resulted in the graph shown in figure 3. In this graph is shown the ratio of number of *C. p. quinquefasciatus* females taken by the lighted trap and the total taken by the lighted and unlighted trap for all nights when both lighted (25-watt) and unlighted traps were run. These ratios are plotted against the days of the lunar cycle with the night of full moon as the centering point. Because of the small numbers of mosquitoes on some days, the days have been grouped by fives. The figure shows that the proportion of females taken by the lighted trap (as compared with the lighted and unlighted trap) was lowest during the periods when moonlight was brightest and was highest when there was little moonlight. This suggests that *C. p. quinquefasciatus* females are attracted to light on dark nights but not necessarily on light ones.

DISCUSSION.—The data presented here indicate that variations owing to position of the trap and to nightly variation are, as was previously believed, very important sources of error in the evaluation of light traps. These are, in fact, so important that unless properly handled, none except the most striking factors can be evaluated. The device used in this study eliminates nightly variation by testing several factors in a single night, and position variation by averaging the effects of position over the four traps. When comparisons are made in which nightly variations are reintroduced, as in the testing of adjacent and opposite designs, the experimental design loses its efficiency and only gross effects can be detected.

The large amount of variation between similar traps placed only 2 to 3 m. apart, which is eliminated when the traps are constantly interchanged, suggests that attraction to a trap may take place over a remarkably short distance. No way has been devised by the writers for determining from what distance mosquitoes are attracted to a light trap but these results and observations recorded in the literature suggest it to be very short.

Attractants are usually repellent at high dosages but the data in this study suggest a linear relationship at the light intensities tested. Further tests are planned to deter-

Table 5.—Numbers of *Culex p. quinquefasciatus* females taken in four experiments on light intensity.

EXPERIMENT	0-WATT	25-WATT	50-WATT	75-WATT
1	4	13	12	6
2	5	11	21	28
3	66	49	39	31
4	34	41	61	74
Total	109	114	133	139

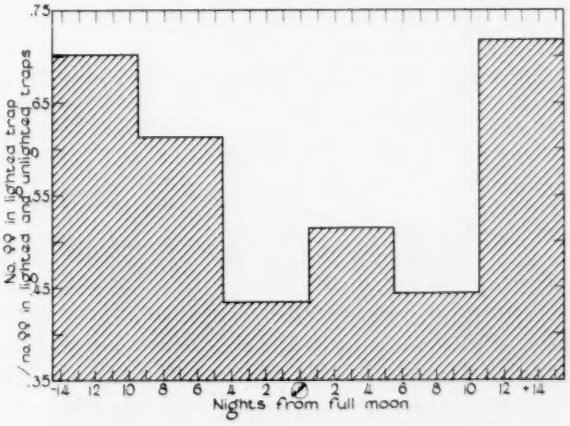

FIG. 3. Ratio of numbers of *C. p. quinquefasciatus* females in lighted trap to total in lighted and unlighted traps as related to periods of the lunar cycle.

mine at what light intensities repellency is noted. This relationship will not be entirely meaningful because of the suction produced by the fan of the trap but it is important from a practical point of view to know how large a bulb can be used without repellency being manifested.

The most interesting aspect of this study is the large number of mosquitoes taken by light traps without lights. This has been previously noted (Headlee 1932, Huffaker & Back 1943, Bargren & Nibley 1956, Reed 1959) but no explanation has been offered for the finding. The results here presented indicate that the unlighted traps caught almost exclusively *Culex* mosquitoes although large numbers of *Aedes* were also present. It is not possible to compare the absolute numbers of *Culex* and *Aedes* mosquitoes present but indications were that the numbers were of the same order of magnitude. It does not seem likely that the number of *Culex* adults in the unlighted trap were just those that happened to be in the volume of air sampled by the traps (Huffaker & Back (1943), however, reached a different conclusion). It therefore seems likely that *Culex* mosquitoes are attracted to unlighted traps as well as to lighted ones. In the case of *C. tarsalis*, light or some other factor associated with the lighted bulb (as heat) increased the attractance of the trap.

Bargren & Nibley (1956) found that the attractance of colored bulbs was blue>yellow and white>red with *Aedes vexans* (Meigen) and *Chaoborus punctipennis* (Say). The same was true with *Culex salinarius* Coquillett except that yellow was more attractive than white. With *Culex nigripalpus* Theobald the four colors were of about equal attractance; with *C. p. quinquefasciatus*, red, yellow, and white were about equally attractive but were more attractive than blue. These findings, as well as the present ones indicate that some *Culex* mosquitoes may get into light traps for different reasons than is the case with *Aedes* mosquitoes. It will be noted that the species taken in unlighted traps are those which utilize shelters as resting places. Some species which rest in vegetation, as *A. nigromaculis*, are not taken in unlighted traps. (*Anopheles walkeri* Theobald, on the other hand, rests in vegetation and is strongly attracted to light.) This suggests that *Culex* mosquitoes may enter light traps while seeking diurnal resting places. Preliminary observations were made on the time that mosquitoes enter the light traps but these are too scanty to be meaningful as yet.

Reed (1959) found that *C. tarsalis* was equally abundant in lighted and unlighted traps and that *C. p. quinquefasciatus* was more abundant in unlighted traps. In the present study in which a more efficient design is utilized, the findings are similar but slightly different. *C. tarsalis* shows a rather low degree of light attraction. *C. p. quinquefasciatus* appears to be slightly attracted to light on dark nights but this may be abolished or possibly even reversed on light ones.

An important finding of this and previous studies is that trapping of *C. p. quinquefasciatus* females can be most conveniently done by using "suction" traps without light. Collections made in this way require much less time to sort because of the general lack of other kinds of insects.

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NOTICE TO AUTHORS

Authors are urged to consult the latest revised list of "Common Names of Insecticides." See JOURNAL OF ECONOMIC ENTOMOLOGY 52(2): 361–362, April 1959 and 52(5): 1032, October, 1959. Reprints are available free upon rtquest to the Office of the Executive Secretary.

The chemical definition of each insecticide, the name of which does not appear in these lists, should be given the first time the name occurs in the abstract and the first time the name occurs in the text of a manuscript accepted for publication by the Entomological Society of America.

Selection and Evaluation of Pea Aphid-Resistant Alfalfa Plants[1]

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ABSTRACT

Various selection criteria were effective in isolating pea aphid, *Macrosiphum pisi* (Harr.), resistant plants from susceptible alfalfa, *Medicago sativa* L., populations and these selection criteria also appeared suitable for the evaluation of progeny from resistant plant selections. Selection of vigorous surviving seedlings after infestation with large aphid populations in early seedling stage was a practical and rapid method for use in the selection of resistant plants. Selection and evaluation for resistance were also accomplished on the basis of differential plant stunting at various growth stages and on basis of aphid colonization on individual plants. Although some of the techniques presented were applicable to field conditions under natural aphid infestation, the advantage of greenhouse screening and evaluation in the seedling stage is evident. The general agreement of the results obtained with the various techniques employed indicates their validity and usefulness as a basis for selecting and evaluating resistant plants.

Various methods for selection and evaluation of alfalfa, *Medicago sativa* L., plants resistant to the pea aphid, *Macrosiphum pisi* (Harr.), have been utilized. Progress in the development of a pea aphid-resistant alfalfa variety depends to a large extent on expedient methods and techniques for initial recognition of resistance and evaluation of selected progeny. It appears that a plant breeding program could be facilitated if suitable methods for mass screening and evaluation in the greenhouse could be evolved to supplement individual-plant evaluation and selection from naturally infested fields.

The principal purpose of this investigation was to determine whether resistant plants could be isolated by infesting alfalfa seedlings with pea aphids and whether seedling techniques could be used as a progeny test to evaluate resistant plants. This paper gives a resume of all methods and techniques used at the Kansas Agricultural Experiment Station to select and evaluate alfalfa plants in various stages of growth.

REVIEW OF LITERATURE.—The pea aphid is a pest of considerable economic importance in production of alfalfa and certain other legumes. Although resistance to this pest was reported in peas by Searls (1932), in alfalfa by Blanchard & Dudley (1934), in vetch by Albrecht (1940), and in red clover by Jewett (1941), insecticides presently provide the primary means of control. Painter & Grandfield (1935) reported differential reactions of alfalfa varieties to a natural infestation of pea aphids in the field. Dahms & Painter (1940) showed that rates of pea aphid reproduction on alfalfa plants selected for resistance in the field were lower than on plants selected for susceptibility, and selfed progeny from these two groups of plants could be distinguished in greenhouse tests. Albrecht & Chamberlain (1941) were able to differentiate F_2 hybrid progenies of resistant and susceptible plants on the basis of per cent seedlings killed; however, a year later all plants appeared susceptible. They concluded that resistance to pea aphids in alfalfa was not a stable character but indicated that a further study should be made on the effect of environment on the expression of resistance, since it appeared that loss of resistance may have been due to poor illumination and occasional low greenhouse temperatures. Dahms & Painter (1940) had previously shown that pea aphids had a lower optimum temperature for reproduction on certain resistant alfalfa plants than on susceptible plants. Emery (1946) attributed temporary immunity to pea aphids in alfalfa to an acid condition in the plant associated with various environmental factors. He observed that it was possible to locate plants having high immunity to pea aphids by growing them under identical conditions and observing the number of aphids present. More recently, Jones *et al.* (1950) presented a factorial interpretation of the inheritance of pea aphid resistance in alfalfa. The survival of a second instar nymph and its reproductive life was recorded for individual plants as a measure of resistance.

Harrington (1945) and Cartier (1959) reported biotypes of pea aphids varying in reaction to resistant and susceptible varieties of peas. Evans & Gyrisco (1956) stated that the *fundatrigenae* and *sexupare* forms of the pea aphid survived and reproduced on alfalfa whereas alfalfa was unsatisfactory for the *aliencolae*.

Painter (1951) discussed methods of studying resistance, divided the resistance mechanism into three categories—preference, antibiosis, tolerance—and indicated the desirability of combining all these factors in an insect-resistant variety. Although pea aphid resistance has been known since 1934, no highly resistant alfalfa variety is available. This suggests that the methods used in developing one may have been inadequate.

MATERIALS AND METHODS.—*Source of Aphids and Plants.*—Field populations of pea aphids were used whenever available for screening and evaluation in greenhouse studies. Cultures on succulent alfalfa and broadbeans (*Vicia fabae*) provided abundant pea aphids which could be used throughout the entire year. Various sources of plants including Buffalo, Ladak, and Vernal were used in this study; however, major emphasis was placed on Cody, because it possessed resistance to spotted alfalfa aphid, *Therioaphis maculata* (Buckton).

Antibiosis Tests.—In antibiosis tests used in conjunction with, or as an evaluation of, different techniques, two methods and types of cages were used. The cage previously described by Hackerott & Harvey (1959) was used to measure pea aphid antibiosis. Five first- or second-instar nymphs were caged on excised stems with a single

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trifoliolate leaf selected from near the terminal of the stem. The resistance of individual plants was based on the number of nymphs that matured and reproduced as recorded on alternate days for a 9-day period. In the other method a cage similar to that described by Peters & Painter (1958) was used. A larger cage was modified to increase the ventilation thereby reducing the humidity owing to transpiration. Eight apterous adults were used as an initial infestation and were caged on the vigorous terminal of a rooted plant. Antibiosis was determined by adult survival and fecundity over a 7-day period.

Tests in Early Seedling Stage.—Seedlings were established in greenhouse flats in rows 2 inches apart and at the rate of approximately 100 per 15-inch row. Scarified seed was used to ensure more uniform emergence. A fungicide and a sand covering aided in reducing damping off. Seedlings in the early cotyledon stage were infested by shaking large numbers of aphids over those in each flat. Additional aphids were added as necessary to maintain a high population, and barriers were used to confine them. Single flats were encircled by a clear plastic sheet 18 inches high, and larger numbers of flats were enclosed by a cage constructed of fine nylon mesh with a supporting wooden framework.

Seedling survival following aphid infestation in the cotyledon stage was evaluated as a technique for selecting resistant plants of susceptible varieties. Cody, Buffalo, Ladak, and topcross progeny of a resistant plant selection (P-42) were screened for survival in a test with 15 replications (1500 seedlings per entry). Fifteen of the most vigorous surviving seedlings from each entry, along with unselected seedlings of the same material, were transplanted to greenhouse pots and subsequently evaluated for antibiosis by caging aphids on excised stems.

Thirty-three plants known to vary in pea aphid resistance as measured by antibiosis were included in a recombination block and approximately 100 seedling progeny of each clone were subjected to the early seedling test to determine parent-progeny relationships. Stunting as a measure of resistance was determined by the number of plants that had attained the cotyledon, unifoliolate, or trifoliolate stage after differences among progenies became apparent. The surviving seedlings were counted after it was evident little additional mortality would occur by prolonging the infestation. Correlations were calculated to determine the relationships between per cent seedlings which had developed trifoliolate leaves and eventual seedling survival; per cent seedlings which had developed only to the cotyledon or unifoliolate stage and per cent seedling survival; number of live nymphs produced in antibiosis tests on parental clones and per cent seedling survival.

Tests in Late Seedling Stage.—Two methods were used to select and evaluate plants in the late seedling stage. In both methods, when plants were approximately a month old, they were clipped at a uniform height of about an inch above the soil level. Plants were uniformly and heavily infested when they had made 2 to 4 inches of regrowth. Repeated reinfestation was generally not required. The aphids were redistributed by brushing about 2 days after the initial infestation to minimize the effects of a nonuniform initial infestation.

In one selection and evaluation method, plants were classified on the basis of aphid numbers when differences became evident in the form of well-established colonies on various individual plants. The seedlings were grown in plant bands to facilitate observation of individual plants. Generally 1 to 2 weeks were required to differentiate between plants on this aphid-colony basis. About 60 Buffalo seedlings previously selected for resistance to the spotted alfalfa aphid were placed in three grades according to pea aphid populations and subsequently tested for antibiosis. In addition, seedling progeny of Vernal and Buffalo selections which had previously been selected for spotted alfalfa aphid resistance were classified according to pea aphid populations, and the plants in Grade I were tested for antibiosis. The plants were classified on the following basis: Grade I had no established colony; Grade III plants had one or more numerous, prolific colonies; Grade II was intermediate and had only a small colony.

A second method relied on the degree of stunting as a selection criterion. Approximately 25,000 seedlings of Cody alfalfa were infested and a similar number were maintained free of aphids in an adjacent greenhouse to serve as a control. After 2 months of aphid infestation, the tallest and most vigorous seedlings that appeared least injured were placed in a nursery space-planted with Cody alfalfa. Thus the progenies from these selected plants may be considered topcrosses. The topcross progenies of 21 of these plants were compared with unselected Cody in an early seedling survival test.

Tests of Mature Plants.—The plants mentioned above were further evaluated in the fall after seed harvest when a low infestation of pea aphids occurred on the succulent regrowth. The fall infestation provided an opportunity to determine natural aphid populations on unselected plants and plants selected in the greenhouse on the basis of lack of stunting. These same selected and unselected plants were transplanted to greenhouse pots and evaluated in a series of antibiosis tests. Excised stems with trifoliolate leaves were used.

In the presence of a natural high field infestation of pea aphids, plants were selected on two criteria. Several thousand plants in a space-planted nursery were evaluated by shaking the plant over a board or pan marked in inch squares and estimating the number of aphids. Twenty-seven plants were also selected on the basis of lack of stunting and damage from farmers' fields under severe outbreak conditions.

In the greenhouse, aphid populations were determined on succulent regrowth of Cody, Buffalo, and Ladak plants. Individual plants were infested with 100 aphids and were evaluated on the basis of aphid colonization after it became evident that more than just temporary or transitory colonies had become established. An additional evaluation of all mature plant selections for resistance was made by antibiosis tests.

RESULTS.—*Early Seedling Stage.* Per cent seedling survivals of plants of three susceptible varieties and a resistant selection are shown in table 1. The topcross progeny of P-42, a plant selected from Cody for resistance in the late seedling stage, survived 72 per cent and Ladak 16 per cent, both of which were significantly better than Cody or Buffalo. Since Cody is resistant and Buffalo susceptible to spotted alfalfa aphids, it appears that pea aphid and spotted alfalfa aphid resistance may be conditioned by

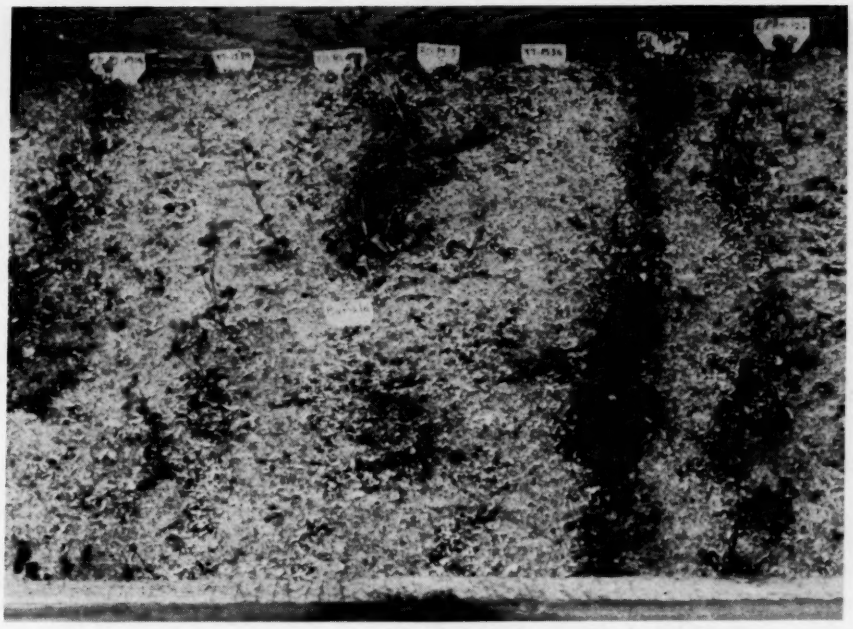

FIG. 1.—Differences in survival of seedling progeny alfalfa plants following a heavy pea aphid infestation in the early cotyledon stage.

independent genetic or physiological factors. The antibiosis of plants selected on the basis of survival and of similar unselected plants is also compared in table 1. There was a marked decrease in nymphal survival on selected surviving plants compared with survival on unselected plants for each of the three varieties as shown by average number of nymphs per plant on the fifth day and by percentages of plants on which at least one aphid matured by the ninth day. Selected and unselected plants of the resistant strain P-42 were about equally as resistant as plants selected for resistance from the varieties.

A comparison of parent-progeny relationships may be made from table 2 which includes seedling survival of progenies from plants known to differ in resistance as measured by antibiosis. Although occasional divergences were great, the correlation between aphid fecundity in

Table 1.—Survival of plants of three alfalfa varieties and topcross progeny of resistant plant, P-42, following pea aphid infestation in early seedling stage and comparative antibiosis reaction of 15 selected and 15 unselected plants from each entry.

Entry	Per Cent Seedling Survival[a]	Antibiosis Reaction: Mean No. of Nymphs on[b] Selected Plants	Mean No. of Nymphs on[b] Unselected Plants	Per Cent Plants on Which Nymphs Matured[c] Selected	Per Cent Plants on Which Nymphs Matured[c] Unselected
Buffalo	3 a	0.1	3.2	6	86
Cody	7 ab	.2	1.6	3	65
Ladak	16 c	.7	2.5	17	70
P-42	72 d	.1	.5	0	10

C. V. = 11.4%

[a] The means with same letter do not differ at 5% level of probability based on Duncan's multiple range test. Analysis of variance based on transformation of data to arcsin scale.

[b] Arithmetic mean for two tests on fifth day after infestation with five nymphs per plant in each test.

[c] Arithmetic mean for two tests on which one or more nymphs matured by ninth day as determined by reproduction.

Table 2.—Antibiosis of parental alfalfa plants and the stunting and survival data of their progeny after infestation with pea aphids in the seedling stage.

Parental Plants[a]	No.[b] Seedlings Tested	Antibiosis Test[c] (No. of nymphs produced)	Seedlings Stunted (%)[d] Cotyledon and Unifoliolate	Seedlings Stunted (%)[d] Trifoliolate	Seedlings Surviving (%)
1	93	12	10.8	68.8	77.4
2	89	8	7.8	75.3	74.2
3	85	1	12.9	71.8	74.1
4	92	0	13.1	83.7	72.8
5	83	38	14.4	63.9	71.1
6	92	13	11.9	69.6	70.7
7	88	23	17.1	67.0	70.5
8	87	13	18.3	58.6	70.1
9	31	13	6.4	45.2	69.8
10	91	3	14.3	69.2	69.2
11	87	42	6.8	69.0	69.0
12	90	2	17.8	70.0	66.7
13	86	24	25.6	60.5	65.1
14	92	0	11.6	59.8	63.0
15	97	3	9.2	62.9	61.9
16	89	39	14.6	60.7	59.6
17	81	2	21.0	68.0	56.8
18	80	25	13.8	65.0	55.0
19	88	3	18.2	67.0	54.5
20	50	75	16.0	58.0	50.0
21	85	15	9.5	62.4	49.4
22	97	175	63.9	11.3	48.5
23	89	41	34.9	33.7	40.4
24	87	273	60.9	20.6	33.3
25	85	95	29.4	24.7	28.2
26	92	41	41.3	22.8	26.1
27	89	270	52.9	23.6	25.8
28	85	180	61.2	8.2	24.7
29	90	190	51.1	13.3	24.4
30	94	215	53.3	18.1	23.4
31	91	175	37.4	15.4	22.0
32	95	125	46.4	11.6	16.8
33	96	200	62.5	12.5	12.5
Susceptible checks	137	163	48.6	2.2	2.9
Resistant checks	134	0	21.1	69.0	78.4

[a] The data for the resistant and susceptible checks are arithmetic means of four entries and were not used in calculating the correlation coefficients.

[b] Stand count was made 3 days after seedling emergence. Subsequent counts were made according to the timetable given in "Materials and Methods."

[c] Number of nymphs produced by eight apterous adult pea aphids during a 7-day period on the parental plants.

[d] Plants were infested 3 days after emergence. Per cent seedlings stunted represents the per cent of young cotyledon seedlings that attained the subsequent growth stages: unifoliolate and trifoliolate.

Table 3.—A comparison of pea aphid resistance grades of alfalfa plants in the late seedling stage and the pea aphid antibiosis of the same plants.

Grade[a]	Per Cent Plants per Grade	Per Cent Plants with Antibiosis Level[b]			Mean No. Live Nymphs Produced[c]
		High	Intermediate	Low	
I	37	40	10	50	26.6
II	25	0	25	75	39.8
III	38	0	0	100	78.1

[a] Grade I had no established colonies; Grade II was intermediate; Grade III had numerous, prolific colonies.

[b] High antibiosis was about equal to a known resistant check; low antibiosis had half the reproduction of a known susceptible check.

[c] Mean number of nymphs produced from initial infestation of 6 apterous adult pea aphids per plant over a 7-day period.

caged leaf tests of parents and seedling survival of progeny was highly significant ($r = +0.80$).

In this test, considerable differentiation was noted among and within seedling progenies in regard to stunting by pea aphids. The parallelism between numbers of seedlings with trifoliolate leaves and eventual seedling survival was striking ($r = +0.91$). A negative correlation of 0.84 was obtained between number of seedlings that had developed only to the cotyledon and unifoliolate stages and seedling survival. The difference in seedling survival following a heavy pea aphid infestation is illustrated in figure 1.

Late Seedling Stage.—The antibiosis of 60 plants previously classified on the basis of aphid numbers per plant is shown in table 3. Forty per cent of the plants in Grade I had a high level of antibiosis, while all plants in Grade III had a low level. The percentage of plants per grade for Vernal and Buffalo selections and the antibiosis of plants in Grade I is presented in table 4. Among 1,150 plants graded, 69 were in Grade I and slightly more than half of these had a high level of antibiosis.

Forty-three plants from 3 to 5 inches taller than the bulk of the 25,000 seedlings were selected from 3-month-old Cody alfalfa seedlings following a 2-month period of pea aphid infestation. Although most of the plants were retarded in growth, few were killed by aphids. A smaller difference in height occurred among the uninfested controls, and most of them were taller than the 43 selected plants. These selected plants were evaluated as described below.

An average of 44% (range 8 to 65) seedling survival was recorded for topcross progeny from Cody plants previously selected for resistance in the late seedling stage on the basis of the lack of stunting compared with 3% seedling survival for progeny from unselected Cody plants. The most resistant progeny was obtained from P-42 (65% survival), which was also used in a previously reported test (table 1). Aphid injury to surviving seedlings ranged from near death to marked stunting, with none of the surviving seedlings being more than half as large as seedlings in the uninfested controls.

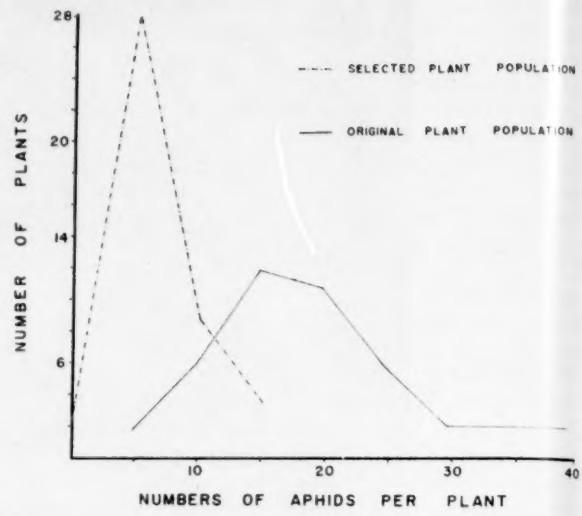

FIG. 2.—Frequency distribution of pea aphids per plant occurring in a low field infestation on plants selected in the late seedling stage for resistance on the basis of lack of stunting and on original unselected plant population

Cody plants, initially selected in the greenhouse on the basis of lack of stunting in the late seedling stage, were evaluated in the field under a natural infestation (fig. 2). The mean number of aphids per resistant plant selection was 4.7 compared with 17.7 for unselected plants as shown in figure 2. This difference was highly significant.

Survival of pea aphid nymphs caged on excised plant stems of the 43 resistant selections compared with unselected plants appears in table 5. Numbers of nymphs on plants selected for resistance declined rapidly compared with those on unselected plants. For example, all introduced nymphs were dead on 67% of the selected plants compared with only 3% on the unselected plants 5 days following infestation. Maturation of nymphs as determined by reproduction occurred on 91% of plants selec-

Table 4.—Alfalfa plants graded for resistance on the basis of pea aphid numbers and antibiosis of Grade I plants.

Origin[a]	No. of Plants Tested	Per Cent Plants in Grade			No. of Grade I Plants	Per Cent of Grade I Plants with Antibiosis Level		
		I	II	III		High	Intermediate	Low
Vernal	160	21.7	26.7	51.6	35	51.9	14.8	33.3
Buffalo	990	3.4	5.7	90.9	34	59.3	20.9	19.7
Total	1,150	—	—	—	69	—	—	—

[a] These plants previously selected for spotted alfalfa aphid resistance had no previous selection for pea aphid resistance. Later tests indicated that one or two of the parental Vernal plants, from which these progeny selections were made, may have possessed a degree of pea aphid resistance.

Table 5.—Comparative antibiosis of 43 alfalfa plants selected on the basis of lack of pea aphid injury in late seedling stage and of 43 unselected plants.

Days After Infestation	No. of Nymphs per Plant[a]		Per Cent of Plants on Which: All Nymphs Died[a]		Per Cent of Plants on Which: One or More Nymphs Matured and Reproduced[a]	
	Selected	Unselected	Selected	Unselected	Selected	Unselected
1	4.2	4.7	2	0	0	0
3	2.1	4.1	18	0	0	0
5	.9	3.6	67	3	0	0
7	.4	3.1	85	7	2	41
9	.2	1.9	90	9	10	91

[a] Arithmetic means for three tests. In each test a single plant leaf was initially infested with five nymphs.

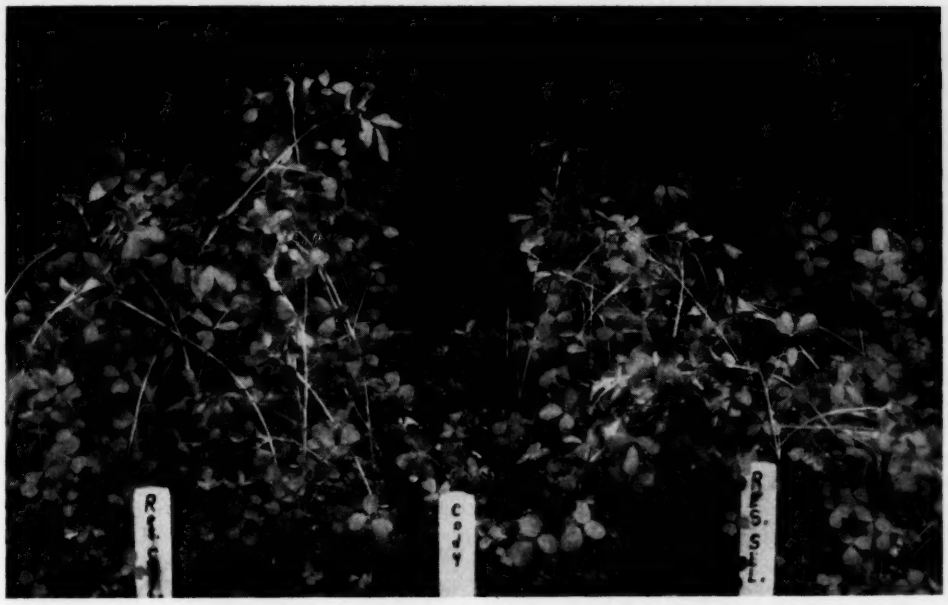

FIG. 3.—Relative stunting of Cody compared with progenies of resistant selections following a heavy pea aphid infestation in the seedling stage.

ted at random compared with only 10% on selected plants.

A comparison of relative stunting following aphid infestation in the late seedling stage of topcross progeny of a resistant plant selection (P-42) and Cody is presented in figure 3.

Mature Plants.—Twenty-five hundred plants in a space-planted nursery were evaluated for pea aphid resistance on the basis of pea aphid numbers per plant. According to this criterion approximately 6% were classified as resistant and 50 of them were brought into the greenhouse for antibiosis tests. Forty-four plants had a high level and six a moderate level of antibiosis.

Twenty-seven plants were selected from farmers' fields which had been heavily damaged. In greenhouse antibiosis tests these plants, which were selected for lack of injury in the field, were classified as follows: 16 high, 7 moderately high, and 3 moderate antibiosis. Those classified as having a moderate level of antibiosis had less than half as many live nymphs as were produced on the susceptible check.

In the greenhouse, aphid populations were determined on 54 Cody and 100 each of Ladak and Buffalo mature potted plants after a uniform level of infestation. Antibiosis tests revealed a high level of resistance in six of eight plants selected from Cody when compared with susceptible checks. The other two plants had a moderately high level of antibiosis. Nine of ten Ladak and two of the ten Buffalo plants selected for low aphid population appeared resistant in antibiosis tests while plants recorded as having a high aphid population all were susceptible.

DISCUSSION.—Preliminary observations indicated that pea aphids transferred from alfalfa and broadbeans were equally suitable for testing alfalfa. The survival and fecundity of aphids reared on broadbeans and transferred to known resistant and susceptible alfalfa were not noticeably different from that for aphids reared on alfalfa.

Pea aphids migrated readily and high concentrations of aphids were required to obtain the desired seedling mortality. The results of a preliminary experiment with a susceptible variety indicated that barriers aided in maintaining the desired concentration and consequent seedling mortality. Earliness of infestation was also an important factor in contributing to the death of susceptible seedlings as observations indicated that emerging seedlings were killed more readily than those infested after unifoliolate leaves had formed. Repeated introductions of generous quantities of pea aphids were required to maintain a high population.

Killing susceptible seedlings in an early stage of growth and retaining the most vigorous survivors appeared to be a practical and rapid method for selecting resistant plants from a predominantly susceptible population. This method was well adapted to mass screening a large number of plants in a relatively small greenhouse area.

The value of the early seedling survival technique as a progeny test and as a measure of the combining ability of the parental resistance was shown in a topcross progeny test. The highly significant negative correlation between numbers of nymphs produced in antibiosis tests on parental clones and per cent seedling survivals of their progeny further demonstrates the utility of early seedling survival as a progeny test. This correlation suggests the heritability of resistance and the relationship between antibiosis and seedling tolerance. However, seedlings selected for resistance that did not appear resistant in antibiosis tests may possess some tolerance to aphids.

Resistant plants may be selected in many stages of growth on the basis of stunting. The highly significant correlation between early seedling stunting and eventual seedling survival indicates that, in the absence of sufficient quantities of pea aphids to effect high seedling mortality, selection and evaluation might be based on early seedling stunting. Early seedling stunting as shown by re-

tardation of growth was readily classified according to growth stages: cotyledon, unifoliolate, and trifoliolate. The general agreement between parental antibiosis of Cody plants selected on the basis of lack of stunting in late seedling stage and early seedling survival of their topcross progeny indicates that this selection criterion was a measurement of resistance.

The importance of the early seedling stage testing was twofold. Selection pressure was exerted on the young seedlings for tolerance to the aphids. A large percentage of the surviving plants also showed high antibiosis.

The high relationships between antibiosis and aphid numbers on individual plants, as expressed by preference, indicates that selection for resistance might be based on actual aphid counts in the absence of damaging populations.

Discrepancies between plants graded on the basis of aphid numbers and their antibiosis reaction were noted. An explanation may be found in the inherent differences between the two test methods. In grading plants, aphids were not confined to a particular plant as they were in antibiosis tests and, therefore, aphids had freedom of movement and plant selection. Caged aphids may have been able to survive and reproduce though actually "preferring" another plant; therefore, these two tests may in part measure two different combinations of plant characteristics. In this manner a third component of resistance, preference, could be evaluated and incorporated.

Selection on the basis of aphid numbers or stunting in the late seedling stage may not be as practical as early seedling selection since more space would be required to screen a given number of seedlings and sometimes precise differences would be more difficult to obtain in the late seedling and mature plant stages. However, in the present tests it was possible to select resistant plants in all stages of development tested. Both greenhouse and field selection could be based on several different criteria. Regardless of method of selection, additional evaluation of selected plants to determine degree of resistance appeared necessary.

The resistance of selected plants was verified by several methods including antibiosis tests, aphid numbers per plant in field, and seedling progeny tests involving both survival and stunting. The agreement among the various techniques employed demonstrates the usefulness of each criterion as a basis for selecting and evaluating resistant plants. The advantage of utilizing various methods of screening and evaluation in a breeding program is that selection pressure can be exerted for the various mechanisms of resistance—preference, antibiosis, and tolerance. Seedling screening techniques, in which selection criteria are based on differences in seedling mortality and stunting at various stages of growth, measures the sum of these mechanisms.

Although Emery (1946) had observed that it was possible to locate plants having resistance to pea aphids by infesting alfalfa plants growing under identical conditions and observing numbers of aphids present, Albrecht & Chamberlain (1941) indicated that this method was subject to much variation and that killing of seedlings was the best measure of relative resistance. As pointed out in the review of the literature, environmental factors are known to affect the expression of resistance of alfalfa plants in the greenhouse. In greenhouse and field tests, in the presence of natural pea aphid populations, conducted over different seasons of the year resistant and susceptible plants were readily separated by either of the aforementioned methods.

Presumably all pea aphids used in these studies were *fundatrigeniae* or more probably *sexuparae* since males and egg-laying females are rarely produced under Kansas conditions. This contrasts strongly with conditions reported by Evans & Gyrisco (1956) for New York. Biotypes comparable with those reported by Harrington (1945) and Cartier (1959) have not been identified in these tests. The aphids used in these studies came from a number of sources and probably included more than one biotype if such exist in Kansas. It remains to be determined whether the resistant selections made will reduce pea aphid injury to alfalfa and pea aphid populations under various conditions and possible exposure to different biotypes and forms of the pea aphid, but there appears to be good evidence that considerable control could be exerted by a pea aphid resistant variety.

Regardless of method of seedling selection, more critical evaluation of selected plants to determine degree of resistance is desirable. Excised terminal stems or plant parts are sometimes more convenient to use for measuring antibiosis than to cage attached plant parts. Excised leaves were also used by Howe & Smith (1957) and Hackerott & Harvey (1959) to differentiate between plants resistant and susceptible to spotted alfalfa aphids. However, Muller (1958) reported that in black aphid, *Aphis* (*Doralis*) *fabae* Scop., reproduction and survival were increased on excised leaves compared with that on rooted plants of field beans, and that differences between a resistant and a susceptible variety were slight on cut leaves but conspicuous on rooted plants. Although in the present study the use of excised plant parts appeared to be a satisfactory technique to distinguish between pea aphid-resistant and -susceptible plants, it appears that a critical evaluation should be made of excised plant parts versus rooted alfalfa plants in evaluating both pea and spotted alfalfa aphid resistance.

The results of these experiments indicate the following procedures for selection and evaluation of pea aphid-resistant alfalfa plants: Mortality and stunting in the early seedling stage appeared to be the best method to use in initial plant selection. If the plant breeder has a particular group of plants in a more advanced growth stage, that have been selected for specific desirable characteristics, selections for pea aphid resistance may be based on aphid numbers or stunting. In the presence of a natural field infestation plants may be evaluated on the basis of the same two criteria. These selections can then be evaluated by the following: antibiosis tests for the individual selections involving caged aphids, and early seedling progeny tests based on stunting and mortality criteria to determine parent-progeny relationship.

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Analysis of Joint Action of Insecticides Against House Flies[1]

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ABSTRACT

To analyze the joint action of two (or more) insecticides, the actual toxicity indexes of the components and their mixture are determined by dosage-mortality curves. The theoretical toxicity of the same mixture is equal to the sum of toxicity indexes calculated from the percentage of each component times its respective toxicity index. Therefore, the joint toxicity or

$$\text{Co-toxicity coefficient of a mixture} = \frac{\text{Actual toxicity index of a mixture}}{\text{Theoretical toxicity index of a mixture}} \times 100$$

A coefficient of a mixture near 100 indicates probability of similar action; independent action usually should give a coefficient less than 100, while a coefficient significantly above 100 strongly indicates synergism. Toxicity significantly less than that of the strongest toxicant alone shows antagonism.

Thirty-nine mixtures each containing two to four insecticides were tested against the house fly (*Musca domestica* L.) and their results were calculated by this method. Generally, chemically related compounds gave similar action, while unrelated compounds gave independent action. 3-(Dimethoxyphosphinyloxy)-N,N-dimethyl-crotonamide (SD 3562) and sesamex showed high synergistic action. However, a mixture of methyl parathion and sesamex indicated significant antagonism.

Since the mixing of chemicals offers many possibilities in the search for better and more potent uses of toxicants, synergism has become a favorite subject for many biologists working on mixtures. Unfortunately, differences in the definition of synergism and the complexity in the evaluation of dosage-mortality relationship have caused large variations in the results of interpretation even for the same set of data.

Synergism was first used in pharmacology for presenting the unusual combined effect of drugs and was defined by Macht (1929) as "the phenomenon exhibited by the combination of two or more drugs in which the pharmacodynamic effect produced by the mixture is not a simple summation of the effects produced by the two or more individual components. Such combinations produce a pharmacological effect of an unexplained nature in that the effect of one of the components may be greatly heightened or potentiated by the other." Bliss (1939) was largely responsible for introducing the statistical calculation of independent action, similar action, and synergism. The mathematical calculation of his independent action and similar action was revised and extended by Finney (1942, 1947), Plackett & Hewlett (1948), and Hewlett & Plackett (1950). Wadley (1945) devised a simple graphical method for testing joint action of toxicants. Recently, joint action has been discussed more thoroughly by Plackett & Hewlett (1952) and a new classification and new methods of calculation were introduced. Horsfall (1945) proposed the "titration design" for testing both synergism and antagonism.

The purpose of this investigation is to find a simple method for the calculation of joint toxicity of various insecticide mixtures expressed in numerical values, with emphasis on those mixtures in which the components are each insecticidal at test concentrations.

EXPERIMENTAL PROCEDURES.—It has been shown by Sun (1950) that the toxicity of one insecticide with respect to a standard can be transferred by simple calculations to a toxicity index based on another standard and that the calculated index is within the variation of one standard

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deviation of the index obtained by direct determination. The above calculation can be made because the toxicity index obtained from the same standard is a common unit of toxicity to the test species. By extending the above principle of calculation, toxicity indexes can also be added to or subtracted from each other in the calculation of toxicity in mixtures. However, similar calculations cannot be applied to results based on per cent mortality or LD50.

Materials.—Purified aldrin (m.p. 103°–104° C.),dieldrin (m.p. 175°–176° C.), α-chlordane (m.p. 101°–102.5° C.), β-chlordane (m.p. 103.5°–105° C.), endrin (m.p. 229°–230° C.), isodrin (m.p. 234°–237° C.), lindane (m.p. 110° C.), DDT (m.p. 107° C.), rotenone and methyl parathion and technical grades of nicotine (alkaloid), pyrethrins (20% concentrate), sesamex [2-(2-ethoxyethoxy)ethyl 3,4-methylenedioxyphenyl acetal of acetaldehyde] and 3-(dimethoxyphosphinyloxy)-N,N-dimethylcrotonamide (SD 3562) were used for tests. The percentage concentration of each toxicant was expressed as grams of active ingredient per 100 ml. of refined kerosene (No. 10) solution.

Test Method.—The house fly, *Musca domestica* L., National Association of Insecticide and Disinfectant Manufacturers (NAIDM) 1948 strain, was reared at 80° ±2° F. and 40% to 50% relative humidity according to the standard NAIDM method (Anon. 1948) and tested under the same conditions by the horizontal tunnel spray method (Sun 1950). Four-day-old flies were thoroughly mixed and properly sampled before counting 100 individuals into each cage. Three cages of flies of mixed sexes were each sprayed with 0.6 ml. of refined kerosene solutions. The deposit was 41 μl. per 100 flies. For each toxicant and each mixture four concentrations were used. The whole series was tested on the same day and repeated on different days. The results were calculated separately for each series tested on the same day and the standard errors were calculated from three or more results. With the exception of some cases, at least three determinations with 3,600 flies each were made for each toxicant or each mixture.

Methods of Calculating Joint Action.—To test the joint action of two insecticides A and B on a certain species of insect, three dosage-mortality curves should be obtained, one for each of A, B and their mixture M. The toxicity indexes (T.I.) are calculated from these LC50's against A (or B) as a standard (Sun 1950). These results may be used for the following calculations only when the test concentrations of the mixture contain the same ratio of A and B.

$$\text{Actual T.I. of mixture M (using A as standard)} = \frac{\text{LC50 of A}}{\text{LC50 of M}} \times 100 \quad (1)$$

The theoretical toxicity of the mixture is equal to the sum of the toxicity indexes calculated from the percentage of A and B and their respective toxicity indexes.

$$\text{Theoretical T.I. of mixture M} = \text{T.I. of A} \times \%\text{ of A in M} + \text{T.I. of B} \times \%\text{ of B in M} \quad (2)$$

From the actual and theoretical toxicity of the mixture, the joint toxicity can be calculated by the following equation.

$$\text{Co-toxicity coefficient of mixture} = \frac{\text{Actual T.I. of a mixture}}{\text{Theoretical T.I. of a mixture}} \times 100 \quad (3)$$

When the co-toxicity coefficient of a mixture is 100, the effect of this mixture indicates probability of similar action. If the mixture gives a coefficient significantly greater than 100, it indicates a synergistic action. An independent action usually should give a coefficient less than 100, but the toxicity of the mixture should be higher than that of either component.

For a mixture containing three or more toxicants the theoretical toxicity index of a mixture would be equal to the sum of the toxicity indexes calculated from the percentage of each component and its toxicity index.

$$\text{Theoretical T.I. of a mixture containing 3 or more toxicants} = \text{T.I. of A} \times \%\text{ of A in M} + \text{T.I. of B} \times \%\text{ of B in M} + \text{T.I. of C} \times \%\text{ of C in M} + \cdots \quad (4)$$

From this result the co-toxicity coefficient for three or more toxicants in a mixture can be calculated the same way as that for two toxicants.

In certain cases when the actual toxicity of a synergist (or antagonist) in the mixture is insignificant, equation 3 may be simplified as follows:

$$\text{Co-toxicity coefficient of a toxicant and a synergist (or antagonist)} = \frac{\text{Actual T.I. of A (in mixture)}}{\text{Theoretical T.I. of A (toxicant)}} \times 100$$

$$= \frac{\dfrac{\text{LC50 of standard}}{\text{LC50 of A (in mixture)}} \times 100}{\dfrac{\text{LC50 of standard}}{\text{LC50 of A}} \times 100} \times 100$$

$$= \frac{\text{LC50 of A}}{\text{LC50 of A (in mixture)}} \times 100 \quad (5)$$

RESULTS AND CONCLUSIONS.—To determine the joint action of two toxicants, aldrin, dieldrin, and a 2:1 mixture of aldrin and dieldrin were tested on house flies. The eye-fitted curves were drawn from the mortality data (table 1) plotted on log-probit paper. At 50% mortality 0.0096% of aldrin is equal to 0.0046% of dieldrin, or aldrin is 0.48 times as toxic as dieldrin. The LC50 for the above aldrin-

Table 1.—Toxicity to house flies of aldrin, dieldrin and their mixtures.

Toxicant (%)		18-Hour Mortality (%)	Average Mortality (%)	Dieldrin Equivalent[a] in Mixture (%)	Interpolated Mortality[a] (%)
Aldrin	Dieldrin				
0.0075	—	33, 36, 37	35.3		
0.01	—	60, 61, 45	55.3		
0.015	—	62, 69, 63	64.7		
0.02	—	86, 93, 87	88.7		
—	0.0035	28, 41, 45	38.0		
—	0.005	66, 50, 45	53.7		
—	0.0075	71, 70, 76	72.3		
—	0.01	84, 86, 79	83.0		
0.00333	0.00167	36, 42, 34	37.3± 4.2[b]	0.00327	34
0.005	0.0025	57, 49, 53	53.0± 4.0[b]	0.0049	53
0.0075	0.00375	85, 63, 71	73.0±11.1[b]	0.00735	72
0.01	0.005	90, 80, 88	86.0± 4.9[b]	0.0098	83

[a] Calculated by a graphic method (Wadley 1945).
[b] Standard deviation.

dieldrin mixture is 0.0068%. From this toxicity ratio the dieldrin equivalents were calculated and interpolated mortalities for the mixtures (table 1) were compared with the actual mortalities by a graphic method (Wadley 1945). The same series of data can also be calculated by the present method. When dieldrin is taken as the standard the toxicity indexes for aldrin and dieldrin are 48 and 100, respectively. Equations 1 and 2 are used for the calculation of the actual and theoretical toxicity index of the mixture and the joint toxicity of the mixture is calculated by equation 3.

Actual T.I. of aldrin-dieldrin mixture

$$= \frac{0.0046\%}{0.0068\%} \times 100 = 67.6$$

Theoretical T.I. of aldrin-dieldrin mixture

$$= 48 \times 66.7\% + 100 \times 33.3\% = 65.3$$

Therefore, co-toxicity coefficient of aldrin-dieldrin mixture

$$= \frac{67.6}{65.3} \times 100 = 103.5$$

Since the co-toxicity coefficient (103.5) of a 2:1 aldrin-dieldrin mixture is very close to 100, the result indicates similar action. The toxicity data were also calculated by a graphic method (Wadley 1945). Results in table 1 indicate that there are no significant differences between the actual and interpolated mortalities. When calculated by the Finney (1942) method, the toxicity of the aldrin-dieldrin mixture also indicates similar action. Since aldrin and dieldrin are closely related compounds, it is reasonable that they should give similar action.

The joint toxicity of three (or more) toxicants may be calculated similarly. Pyrethrins, β-chlordane and lindane were tested separately and as mixtures containing 69% pyrethrins, 20.7% β-chlordane and 10.3% lindane. The LC50's and toxicity indexes are shown in table 2. From these data the theoretical toxicity and the co-toxicity coefficient were calculated by equations 4 and 3, respectively.

Theoretical T.I. of mixture

$$= 100 \times 69\% + 310 \times 20.7\% + 857 \times 10.3\% = 221$$

Co-toxicity coefficient of pyrethrins-β-chlordane-lindane mixture

$$= \frac{114}{221} \times 100 = 51.6$$

Results of calculation indicate that the co-toxicity coefficient (51.6) of the mixture is much lower than that for similar action (*i.e.*, 100) and that the actual toxicity index of the mixture is higher than that of any component alone. The effect of such combination indicates an independent action. Since pyrethrins, β-chlordane and lindane are not closely related compounds, it is expected that independent action occurs.

Table 2.—Toxicity to house flies of pyrethrins, β-chlordane, lindane and their mixtures.

Toxicant	LC50 (%)	Toxicity Index Obtained from Actual Determination
Pyrethrins	0.066	100
β-Chlordane	0.0213	310
Lindane	0.0077	857
Mixture of pyrethrins, β-chlordane and lindane	0.058	114

Table 3.—Toxicity to house flies of SD 3562, methyl parathion, and their mixtures containing sesamex.

Spray Solution		LC50 of Toxicant (%)
Toxicant	Synergist	
SD 3562	—	0.059
SD 3562	1% sesamex[a]	0.0030
Methyl parathion	—	0.0055
Methyl parathion	1% sesamex[a]	0.015

[a] 1% sesamex solution is not more toxic than kerosene alone.

To calculate the joint toxicity of a mixture containing a synergist (or an antagonist) which is not toxic at the test concentrations, equation 5 may be used. For example, SD 3562 and sesamex were tested separately and in mixtures containing 1% sesamex and various amounts of SD 3562. Repeated tests show that 1% sesamex is no more toxic than kerosene alone. From the LC50's in table 3, the co-toxicity coefficient was calculated by equation 5.

Co-toxicity coefficient of SD 3562-sesamex mixture

$$= \frac{0.059\%}{0.0030\%} \times 100 = 1967$$

In another case, methyl parathion and sesamex were tested separately and in mixtures containing 1% sesamex and various amounts of methyl parathion. The LC50's were obtained and listed in table 3. Equation 5 was also used for the calculation of their joint toxicity.

Co-toxicity coefficient of methyl parathion-sesamex mixture

$$= \frac{0.0055\%}{0.015\%} \times 100 = 37$$

Results of calculation show that the addition of 1% sesamex to SD 3562 increased the toxicity to house flies 19.7 times, indicating high synergism, and that the same amount of sesamex decreased the toxicity of methyl parathion to 37%, indicating a significant antagonistic action. It is an unusual observation that a compound is capable of synergizing one toxicant and antagonizing another, both of which are cholinesterase inhibitors. The study of their mode of action has been reported in another paper (Sun & Johnson 1960).

Without going into the details of experimental data and calculations, the co-toxicity coefficients of 37 mixtures of insecticides are listed in table 4. For mixtures of two closely related compounds, such as aldrin, dieldrin, endrin, isodrin, α-chlordane and β-chlordane, the average co-toxicity coefficient of each mixture, with at least three determinations, varies from 90.2 to 108.6, not more than one standard deviation from 100. It indicates that these compounds have the same or similar mode of action. Their combinations against house flies show little gain or loss of toxicity.

For two less related chlorinated compounds (table 4, II), such as aldrin, dieldrin, α-chlordane or β-chlordane in combination with either DDT or lindane, the co-toxicity coefficients are somewhat less than those of group I. This

Table 4.—Co-toxicity coefficients of 37 mixtures of insecticides tested on house flies.

PERCENTAGE COMPOSITION OF TOXICANTS IN MIXTURES	CO-TOXICITY COEFFICIENT FOR EACH DETERMINATION	MEAN±S.E.
I. Mixtures of closely related chlorinated hydrocarbon insecticides		
33.3% dieldrin+66.7% aldrin	110, 108, 104, 97.8	105 ±2.7
6.25% dieldrin+93.75% α-chlordane	94.7, 106, 94.8	98.5±3.8
25% dieldrin+75% β-chlordane	111, 71.7, 93.7, 82.8, 91.6	90.2±6.5
10% aldrin+90% α-chlordane	126, 118, 94.4, 117, 96.9, 99.4	108.6±5.4
40% aldrin+60% β-chlordane	123, 91.2, 101	105 ±9.4
40% aldrin+60% isodrin	112, 114	113
83.3% α-chlordane+16.7% β-chlordane	110, 118, 102	106.7±5.7
50% aldrin+50% endrin	104, 133	118
II. Mixtures of two less related chlorinated hydrocarbon insecticides		
10% dieldrin+90% DDT	103, 85.8, 101	96.6±5.4
16.7% aldrin+83.3% DDT	115, 81.7, 99	98.6±9.7
60% α-chlordane+40% DDT	106, 105, 102	104 ±1.2
23.1% β-chlordane+76.9% DDT	103, 83.7, 65.7, 88.2	85.1±7.7
40% dieldrin+60% lindane	69.9, 87.4, 76.3, 88	80.4±4.3
57% aldrin+43% lindane	90, 86.2, 69	81.7±6.5
90.9% α-chlordane+9.1% lindane	91.1, 86.3, 110	95.8±7.2
66.7% β-chlordane+33.3% lindane	87.3, 76.2, 79.3	80.9±3.3
87% DDT+13% lindane	91.6, 98.0, 82.2	90.6±4.6
III. Mixtures of two unrelated insecticides		
9.1% dieldrin+90.9% pyrethrins	69.1, 74.3, 58.3	67.2±4.7
16.6% aldrin+83.4% pyrethrins	62.3, 72.2, 68.3	67.6±2.9
60% α-chlordane+40% pyrethrins	57.5, 66, 70.2	64.6±3.8
23.1% β-chlordane+76.9% pyrethrins	79.9, 55.9, 70.9, 63.8	67.6±5.1
50% DDT+50% pyrethrins	74.4, 64.1, 65.9	68.1±3.2
13% lindane+87% pyrethrins	68.2, 70.7, 80.8	73.2±3.9
11.1% dieldrin+88.9% rotenone	57.8, 74.6, 69.2	67.2±5.0
20% aldrin+80% rotenone	58.1, 75.5, 70.4	68.0±5.1
65.2% α-chlordane+34.8% rotenone	81.7, 62.5, 59.5	67.9±6.9
27.3% β-chlordane+72.7% rotenone	50.5, 72.9, 66.4	63.3±6.6
55.6% DDT+44.4% rotenone	64.4, 71.1, 73.6	69.7±2.8
15.8% lindane+84.2% rotenone	84.4, 76.4, 76.4	79.1±2.7
55.5% pyrethrins+44.5% rotenone	108, 86.2, 90.4, 71.8, 88.5	89.0±5.8
0.333% dieldrin+99.7% nicotine	77	77
IV. Mixtures of three unrelated insecticides		
51.3% DDT+41% rotenone+7.7% lindane	64.2, 62	63.1
69% pyrethrins+20.7% β-chlordane+10.3% lindane	66.4, 51.6	59
80% pyrethrins+12% lindane+8% dieldrin	53.8	53.8
51.3% DDT+41% rotenone+7.7% lindane	61.1	61.1
V. Mixtures of three or four related insecticides		
83.3% α-chlordane+11.1% aldrin+5.6% dieldrin	95.0, 97.0, 115	102 ±6.4
71.4% α-chlordane+14.3% β-chlordane+9.54% aldrin+4.76% dieldrin	95.6, 98, 101	98.2±1.6

indicates that the components of these combinations may not be the same in their mode of action, but that they are not entirely different.

Results of mixtures in group III (table 4) show significant lower toxicities. With the exception of the pyrethrins-rotenone combination, the average co-toxicity coefficients vary from 63.3 to 79.1. Approximately one-third of the theoretical toxicity was lost through most of the combinations. Similarly, the same losses of toxicity were obtained with mixtures containing three relatively unrelated insecticides (table 4, IV). These results indicate differences in their mode of action.

One mixture containing three related compounds (α-chlordane, aldrin and dieldrin) and another mixture containing four related compounds (α-chlordane, β-chlordane, aldrin and dieldrin) gave coefficients of 102 and 98.2 respectively (table 4, V). The joint action of these mixtures should be classified as similar action.

Selected data in the literature which contain enough data for the same type of analyses were calculated for their co-toxicity coefficients. Results in table 5 indicate a general agreement between the authors' conclusion and co-toxicity coefficients. In one case the author (Wadley 1945) indicates slightly higher results than similar action; the co-toxicity coefficient (118) is also slightly higher than 100.

DISCUSSION.—The determination of joint toxicity of insecticides is a complicated problem. A simple additive effect based on the mortalities produced by the components applied separately and jointly is rather misleading, because the linear relationship between dosage and mortality is not based on an arithmetic scale but on a log-probit scale. If the toxicity of a mixture is calculated by a simple additive effect, apparent synergistic action can often be found by designing an experiment so that the concentration of each of two (or more) components in the mixture is so adjusted to produce a low but significant mortality (5% to 15%).

Table 5.—Calculation of co-toxicity coefficients from published data.

COMPOSITION OF MIXTURES	TEST METHOD	TEST INSECT	AUTHOR'S CONCLUSION	CO-TOXICITY[a] COEFFICIENT AND S.E.	REFERENCE
Sulfur dioxide and acetone of 5 proportions	Fumigation	Confused flour beetle	Similar action	82.4±2.9	Swisher (1944)
Sulfur dioxide and ethylene oxide of 2 proportions	Fumigation	Confused flour beetle	Similar action	81.5	Swisher (1944)
Rotenone and deguelin	Spray	Aphids	Similar action[b]	118	Wadley (1945)
Parathion and methyl parathion mixtures of 3 proportions	Spray	House fly	Similar action	95 ±1.7	Gersdorff & Mitlin (1951)

[a] Calculated by equations 1, 2 and 3.
[b] Slightly higher than similar action, but not significant.

The statistical analyses for joint action devised by Bliss (1939), Finney (1942), have a sound basis for analyzing joint action of toxicants, but they are rather complex and time consuming for general use. The graphic method devised by Wadley (1945) is not only simple but also agrees with the analysis calculated by Finney's method.

The present method of calculation of joint toxicity is not only simple but also gives an approximation of the gain or loss of toxicity as well as the type of joint action for a mixture. In order to carry out the experiments on joint action of toxicants successfully the following precautions should be observed.

Since a high degree of accuracy is required for the determination of joint action of insecticide mixtures, it is necessary to reduce variation as much as possible. Biological variation which is one of the important factors is occasionally overlooked.

To reduce biological variation it is best not only to have an adequate number of replicates with enough individuals in each replicate, but also to use a species at a certain stage and age giving the least variation. For example, the larval stage usually gives more variation than the adult stage. Insects with a more uniform rate of development, such as the house fly, usually give less variation in susceptibility than those with variable rates of development, such as cockroaches. If several cultures have to be used, it is necessary to mix and sample the insects properly.

Selection of a suitable test method is also important to properly analyze joint action. Methods, such as dry-film, baits, et cetera, are not often reliable, because the test insects may be attracted or repelled by one of the toxicants in the mixture, and any unusual increase or decrease in toxicity may be owing to the difference in pickup or in feeding. Topical application, injection or direct spray methods should give a reasonable accuracy in the application of toxicants to insects. Although the topical application and injection methods have the advantage of giving an individual dose to each insect, only a small number of individuals is often used for each concentration because these methods are more time-consuming. In a long series of experiments all tests may not be completed on the same day. To compensate for the daily variation of insects it is necessary to test each concentration of all toxicants and their mixtures every day with a few insects and the whole experiment should be repeated on different days. On the other hand, a direct spray method does not give the same dosage to each test insect, but it is reasonably accurate and reproducible and a long series of experiments can be completed in 1 day. The average coefficient of variation for the spray method (Sun 1950) used for this study is ±5.8% for 27 determinations of the toxicity of aldrin and dieldrin.

Results on the study of joint toxicity expressed as co-toxicity coefficient can be used not only for the evaluation of joint action of insecticide mixtures in the laboratory but also for the estimation of dosage of each toxicant in insecticide mixtures for preliminary field tests. For example, when two different types of insect pests are present on the same crop at the same time, two kinds of insecticides are often used for their control. Although each toxicant in the mixture is used for control of a special type of pest it may also have some effectiveness against another type. If one has obtained the data on the effectiveness of each toxicant against each pest the toxicant needed in the mixture should be less than the dosage when applied alone. By means of calculating their joint toxicity an approximate ratio of toxicants can be obtained for preliminary field tests with new combinations.

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Designing Orchard Experiments for European Red Mite Control[1]

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ABSTRACT

Experiments based on preventive programs have created a need for satisfactory designs to evaluate acaricides under orchard conditions and to permit statistical summarization and interpretation of the results. The literature regarding mite sampling is briefly reviewed and two examples of preventive experiments and one eradicant type experiment are presented with statistical treatment and interpretation of results.

Data from orchard mite control experiments, in which counts were taken periodically during the season, were analyzed statistically as split-block designs. Before analysis of variance could be conducted, a logarithmic transformation was necessary to remove the proportional relationship between the means and the standard deviations to meet the requirements of an F test. By increasing the number of trees a greater gain in detecting treatment differences was produced than by increasing the number of leaves sampled or the number of sampling dates.

In an example using mild acaricides in a preventive schedule, it was found that the addition of 1 ounce of ovex in each spray to a DDT-parathion formulation gave superior control of the European red mite, *Panonychus ulmi* (Koch). Glyodin used in place of captan also significantly improved control. A DDT-Diazinon (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate) tank mix was as effective as most DDT-parathion formulations. Differences in the other experiments represented were large in one and small in the other, but were not significant in either.

A large number of European red mite (*Panonychus ulmi* (Koch) = *Metatetranychus ulmi* (Koch)) control experiments reported in the literature have dealt with high mite populations. The application of acaricides in many of these experiments has coincided with the common but unpredictable natural declines and fluctuations, making interpretation of results difficult, if not impossible, as the results are frequently based on per cent reduction of mites per leaf. Cutright (1951), in discussing late-season control, indicates that many growers do not recognize these natural declines and that much money, effort, and materials are wasted in control efforts after the damage has already occurred. Boulanger (1958), in studying the physiological effects of European red mite feeding, concluded that severe injury is primarily a function of time. Although he found the number of mites per leaf of importance, the time when these numbers occur was more important.

Mite control experiments based on prevention rather than eradication after the leaves show visible evidence of injury have been used on a limited scale (Lathrop & Hilborn 1950, Kriner *et al.* 1952, Lienk 1955, 1957, Curtis *et al.* 1956). Experiments based on preventive programs have created a need for a satisfactory experimental design to evaluate acaricides under orchard conditions that would permit a statistical summarization and interpretation of the results. It is the purpose of this paper to review the literature regarding statistical aspects of European red mite count data and to present, interpret, and summarize, with the aid of common statistical procedures, the results of preventive-type mite-control experiments.

One of the primary problems in mite-control experiments is presented by sampling and sample size. A wide variety of sampling methods has been used to estimate mite populations on leaves. The objective of most methods has been to increase the sample size to reduce the large variability between samples and to reduce the amount of tedious work required to count dense mite populations. Venables & Dennys (1941) developed a paper imprint method, which found wide use in England. A method of estimating mite populations on a per unit area of leaf surface was used by Baten & Hutson (1943). This method entailed use of a folded piece of cardboard with windows cut in each side, counting the mites with a hand lens. The Mite Brushing Machine was invented by Henderson & McBurnie (1943) and has had considerable acceptance in both England and America. It was found by Chant & Muir (1954) to be more efficient than the paper imprint method, but both these methods were found inferior to direct visual counting of mites and eggs. Morgan *et al.* (1955) classified and critically evaluated most of the common methods used and found the mite brushing machine more efficient for estimating populations of phytophagous and predacious mites than microscopic examination of the leaves.

Although considerable work has been done in developing methods of estimating mite populations on foliage, very little work has been done in determining the number of leaves needed to detect differences between treatments. Baten & Hutson (1943) suggested the use of a 300-leaf sample with their method of counting. Oakland (1953) found that increasing the number of replicates (blocks)

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Table 1.—Spray materials and concentrations used in European red mite Experiment I.

Treatment	Formulation	Material[a]	Rate (lb./100 gal.)	Active Ingredient (oz./100 gal.)
1	145	DDT[b]	2.5	10.0
		Parathion		1.2
		Captan	2.0	10.
2	253	DDT	2.5	10.0
		Parathion		1.2
		Captan	2.0	10.
3	145	DDT	2.5	10.0
	K-101	Parathion		1.2
		Ovex	0.12	1.0
		Captan	2.0	10.
4	145	DDT	2.5	10.0
	Crag 341	Parathion		1.2
		Glyodin	1–1.5	5.4–8.2
5	508 (1957)	DDT[c]	1.5	10.0
		Parathion		1.2
		Captan	2.0	10.
6	508 (1958)	DDT[c]	1.5	10.0
		Parathion		1.2
		Captan	2.0	10.
7	Tank-Mix[d]	DDT	1.5	10.0
		Diazinon	0.33	1.2
		Captan	2.0	10.

[a] All chemicals except glyodin, Diazinon and captan were supplied by Diamond Black Leaf Chemical Company.
[b] Formulations in treatment 1 to 4 consisted of 25% DDT and 3% parathion on the same base of neutralized tobacco stems.
[c] Formulations in treatments 5 and 6 consisted of 50% DDT and 6% parathion formulated on the same base of clay. Treatment 5 manufactured in 1957, treatment 6 in 1958.
[d] A tank mix of 50% DDT and 25% Diazinon.

would result in greater precision in detecting differences between treatments than increasing the number of leaves or the trees per plot. He noted that 10 leaves from each of 4 trees would give the same precision as 25 leaves from each of 3 trees.

For the purposes of this study three mite-control experiments have been chosen to demonstrate a method of handling mite data with common statistical procedures. Two of the examples are experiments in which mild or low concentrations of acaricides were used in preventive type programs, and the third example is from an eradicant type control experiment.

EXPERIMENT I.[3]—*Materials and Methods.*—Three blocks of single tree plots of 10- to 20-year old McIntosh apple trees in an orchard located in Wayne County, New York, were used to evaluate the effectiveness of seven treatments. The treatments were applied with a high-pressure sprayer operated at 550 p.s.i. using a hand-operated trigger gun. The materials used in each treatment are listed in table 1. The number and time of applications are given in table 2. Since all treatments were to be compared with a standard or among themselves, two trees were selected from outside the experimental plots for observation of natural population fluctuations.

Twenty mature leaves from fruit spurs were taken at a 4- to 6-foot height from each of the 21 trees nine times

Table 2.—Spray schedule followed in European red mite experiment, and rainfall between cover sprays, Experiment I.

Spray Schedule		Days Between Sprays	Inches of Rainfall Between Sprays[a]
June 11	First cover	—	—
June 18	Fungicide	7	0.50
June 29	Second cover	17	0.50
July 17	Third cover	19	1.21
August 1	Fungicide	24	0.00
August 7	Fourth cover	21	0.00

[a] From rain gauge located near orchard.

during the season. Ten leaves were taken from the center and from the outside periphery of each tree. Each sample was placed in a half-pint ice cream carton, and the number of eggs and mites (all motile forms) were counted the same or the following day. Direct visual counts were made using a binocular microscope.

The mites encountered in this study were predominantly European red mites. The entire experiment was sampled nine times between June 27 and August 28. The samples were taken 2 days before, 2 days after, and 7 days after the second, third, and fourth cover sprays. The samples collected on each of the 9 dates were regarded as subsamples of each treatment, making 27 replicates of 20 leaves each from each treatment. Because the treatments and blocks remained the same during the experiment, (*i.e.*, were not re-randomized within each sampling date) the experimental design is that appropriate to a split-block design. Although it is common practice either to take samples several times from the same plots throughout the season, or several times following the application of spray materials, it appears that the possibility of regarding these samples as subblocks in a split-block design has been overlooked. Because the egg and mite counts were recorded separately and each furnishes a criterion of the effectiveness of the treatments, the entire experiment may also be regarded as a split-split-block design, which may often yield valuable information on the interaction of eggs versus mites with treatments and dates. However, the computations required to complete an analysis of variance of a split-split-block design are more complex and more difficult to interpret. Separate analyses of the egg and of the mite data were performed for simplicity of presentation and because a split-split-block analysis yielded approximately the same information.

Results.—Before conducting the analyses of variance it was found that the standard deviations of each treatment were proportional to their means (fig. 1). Such a relationship is an indication that the data are not normally distributed and require transformation before conducting an analysis of variance. This fact is not unusual with entomological count data.

Williams (1937) suggested the transformation log $(x+1)$ be used when the means and standard deviations were highly correlated. Austin & Massee (1947) thought that the transformation log $(2x+1)$ as suggested by Pearce (1945) would have been a better transformation than log $(x+1)$ for their European red mite data. Austin and Massee found no single transformation capable of

[3] Work on this experiment was supported in part by a Diamond Black Leaf Chemical Company fellowship grant.

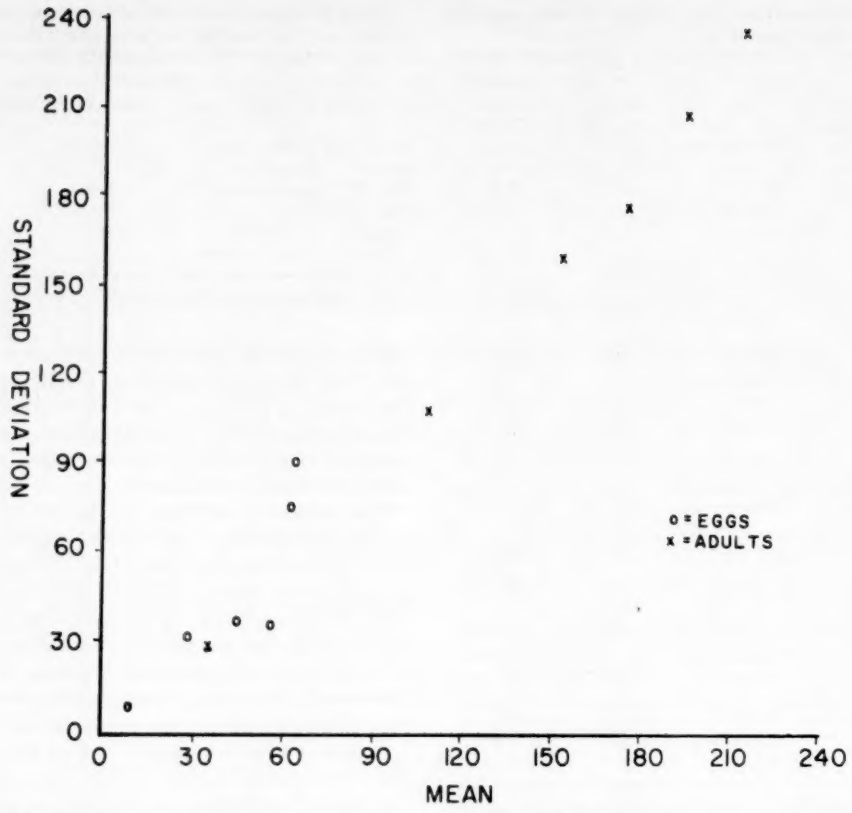

FIG. 1.—A graph showing the relationship of the treatment means to the standard deviations of untransformed European red mite count data from Experiment I. Each point was computed from 27 samples of 20 leaves each.

completely equalizing the contributions from the different sources of variance. They recommended the design of experiments so as to permit separation of the error sums of squares into components, each appropriate to a comparison in which the experimenter is interested. Hueck (1953) plotted the frequency distribution of the number of mite eggs per leaf for three sampling dates. He showed that as the number of mites per leaf increased the distribution approached the normal, whereas with few mites per leaf the distribution was more skewed than a Poisson distribution with the same mean. Hueck found that if counts were expressed as numbers per unit area of leaf, then the distribution approached the Poisson. Oakland (1953) found that the transformation log $(x+1)$ was the most appropriate transformation for his European red mite data. Freeman & Hall (1954) used the transformation log $(x+c)$ where c was a constant that varied from time to time. However, in their data Freeman and Hall substituted $\frac{3}{8}$ for c in this formula. These authors state that the theory behind the use of this transformation was:

"... if all leaves were equally available to the mites, and the sample leaves taken completely at random, the variability of the observed distribution, as measured by its variance, would be expected to be equal to the mean. The distribution in which this occurs is of the Poisson type. However, in practice, disturbing factors are inevitable, both because different types of leaf are not equally available to the mite, and because it is physically impossible to select leaves wholly at random. These disturbances have so large an effect that there is a much greater agglomeration of red spider on some leaves than can be accounted for by a Poisson distribution. In fact, the assumption has been made that the variance is proportional to the square of the mean, in which case the population follows the log-normal distribution. This is the extreme case and only one justified when the disturbing factors have entirely swamped those tending to make the distribution of the Poisson type. More often both factors operate, and such intermediate cases can be regarded as falling into the negative binominal distribution."

Freeman and Hall computed estimates of k values, $k=\bar{x}^2/(s^2-\bar{x})$, to determine at what position on the negative binominal distribution a given set of red mite data lies. They found k values ranging from 0.094 to 1.984, positively correlated with time, thus supporting the findings of Hueck (1953). Anscombe (1948) suggested that if k is less than 2, or if the mean is large with a slightly larger k value, the transformation log $(x+\frac{3}{8})$ be used. Bartlett (1947), in discussing the use of transformations, suggested that log $(x+1)$, or log x be used when the means were proportional to the standard deviation.

Because the means and standard deviations were approximately proportional (fig. 1) the data from this experiment were transformed to log $(x+1)$, where x was the observed number of eggs or adults per 20 leaf sample. This transformation reduced Bartlett's chi square for homogeneity of variances (Snedecor 1956) from 16.60 to 5.54 for the egg and from 44.08 to 2.25 for the mite data (chi square 6 d.f., 1% level=16.81). Replotting the transformed data showed that the proportional relationship

Table 3.—Analyses of variance of transformed European red mite data, Experiment I.

Source of Variation	d.f.	Mean Square[a] Eggs	Mean Square[a] Mites
Blocks	2	0.1067 n.s.	0.5875 n.s.
Treatments	6	2.0239**	2.1177**
Blocks×Treat. (Error *a*)	12	0.3483	0.3443
Dates	8	4.5[illegible]	3.4635*
Dates×Blocks (Error *b*)	16	0.2[illegible]	0.1672**
Dates×Treatments	48	0.1050 n.s.	0.1138**
Dates×Treat.×Blocks (Error *c*)	96	0.0963	0.0624

Results of Duncan's Test[b]

Treatment	Means of Eggs Number	Means of Eggs Log of Nos.	Means of Mites Number	Means of Mites Log of Nos.
1. 145	95.0	1.976 a	42.6	1.629 c
2. 253	67.9	1.832 a	36.4	1.561 bc
3. 145-ovex	14.9	1.172 b	6.1	0.785 a
4. 145-glyodin	62.9	1.798 a	17.4	1.241 b
5. 508—1958	78.8	1.896 a	27.8	1.443 bc
6. 508—1957	66.8	1.825 a	22.4	1.351 bc
7. Tank Mix (DDT-Diazinon)	85.2	1.971 a	29.4	1.468 bc

[a] n.s. not significant, * significant at 5% level, ** significant at 1% level.
[b] Treatment means followed by different letters are significantly different.

between the means and standard deviations no longer existed. On the basis of these data and on similar results of other workers, it appears that the transformation log $(x+1)$ sufficiently normalized European red mite count data to meet the requirements of an F test. Separate analyses of variance were then conducted on the egg and the mite data of this experiment according to standard procedures for a split-block design (Federer 1955). The analyses of variance and the results of Duncan's test (Duncan 1955) for the separation of means are presented in table 3.

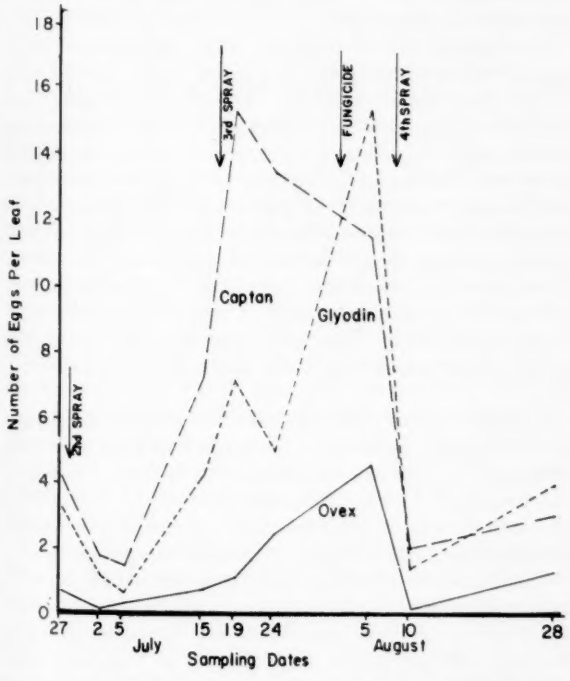

FIG. 2.—The effect of captan, ovex, and glyodin (treatments 1, 3, and 4) on populations of eggs of the European red mite. Treatment 3 differed significantly from all other treatments, while the differences among the remaining treatments were not significant.

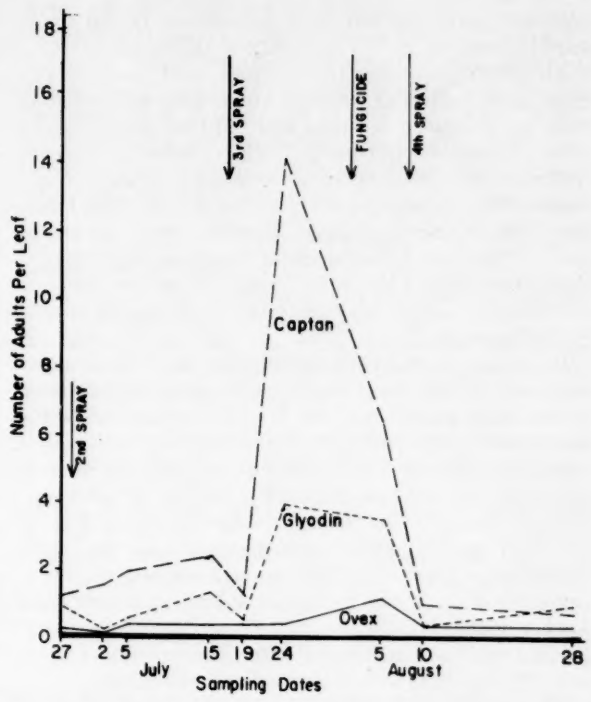

FIG. 3.—The effect of captan, ovex, and glyodin (treatments 1, 3, and 4) on populations of motile forms of the European red mite. Treatments 1, 3, and 4 differed significantly from one another, while the remaining treatments did not differ significantly from treatment 1.

During the summer it was observed that the trees receiving glyodin had somewhat lower mite populations than those receiving captan. The mite populations on the ovex-treated trees remained at a consistently low level throughout the season. Because these two treatments were of special interest and directly comparable to treatment 1 (same insecticide formulation) the means of treatments 1, 3, and 4 were computed[4] for each sampling date for the eggs and for the mites and the values plotted. These results are presented in figures 2 and 3.

Discussion.—In assembling the raw data it was observed that each block or tree had a successive peak and decline of mites in an orderly sequence from west to east as the season developed. This observation was borne out by the significant Blocks×Dates mean squares for both egg and mite data. The nonsignificant Dates×Treatments mean square for the eggs and significant Dates×Treatments mean square for mites was believed to have been owing to the difference in effectiveness of the treatments to the eggs and mites, and to the lack of a definite relationship between the egg and mite population fluctuations. The mite populations on the different treatments reached peak densities and declined on different dates. This sequence of events to a smaller degree was also observed with the eggs. The peak egg populations on the seven treatments were more closely synchronized than were the adult population peaks. Nevertheless, both the

[4] Because the original counts were transformed to logs the correct means are the antilogs of the means of the logs.

eggs and mites reached peak populations on all treatments during the period from July 19 to August 5.

The DDT-parathion formulations used have usually given good control of common orchard insects including both the European red mite and the two-spotted spider mite, (*Tetranychus telarius* (L.)) when applied at this concentration on a 10-day preventive schedule (Dean & Newcomer 1948, McAlister 1949, Newton & List 1949, Hamilton 1950, Kriner *et al.* 1952). However, either an extension of the time between cover sprays or a prolonged period between the last cover spray and harvest has usually favored a mite buildup, often necessitating special control measures.

It was also noted that the application of these spray materials did not always result in an immediate decrease in the adult population (fig. 3). The application of the third cover spray resulted in only a slight dip in the adult population, followed by an almost explosive incresae in numbers. This increase in adults is thought to have been owing to the effect on the egg production of this mite of the DDT applied in the second spray. It has been established that contact with DDT causes a marked increase in egg production in the European red mite (Hueck 1953, Locher 1958). Hueck also lists numerous references reporting similar observations with other species of mites and arthropods as well as with other insecticides.

The addition of glyodin to the 145 formulation of DDT-parathion significantly improved the control of the motile forms (mites) of the European red mite, but not the eggs. The use of glyodin resulted in a suppression of early-season European red mite buildup, but did not entirely prevent it (figs. 2 and 3). These results are similar to those obtained by Hilborn & Lathrop (1950, 1951), Parent *et al.* (1956), Daines (1958), Morgan *et al.* (1958) and others (Ackerson 1958). Clancy & McAlister (1956) demonstrated that 1 to 1.5 pints of glyodin per 100 gallons used at frequent intervals retarded early-season red mite buildup but did not prevent it. They attributed this retardation to the nontoxicity of glyodin to predators of the European red mite. The results of this experiment seem to indicate that glyodin acts only on the motile stages of the European red mite but do not indicate whether this result is the influence of predators or of some other factor. The DDT-Diazinon (*O,O,*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate) treatment was as effective as many of the DDT-parathion formulations and was not significantly different from the 145-glyodin treatment (treatment 4) in the control of motile forms of the European red mite. The 145-ovex treatment (treatment 3) was significantly superior to all other treatments in the control of eggs and adults.

EXPERIMENT II.[3]—*Materials and Methods.*—This experiment was conducted simultaneously with Experiment I on McIntosh trees adjacent to it. Three blocks of single tree plots were used to evaluate the effectiveness of two concentrations, 0.25 and 1 pound per 100 gallons, of a stabilizer[5] in controlling European red mites. The insecticides were DDT and parathion formulated on tobacco base (145 formulation). Glyodin was applied on the same dates and at the same concentrations as used in Experiment I. The method of application, equipment, sampling procedures et cetera were identical with those used in Experiment I.

Table 4.—Analyses of variance of transformed European red mite data, Experiment II.

Source of Variation	d.f.	Mean Square[a] Eggs	Mean Square[a] Mites
Blocks	2	0.3083 n.s.	0.0181 n.s.
Treatments	1	0.0720 n.s.	0.3838 n.s.
Blocks×Treatments (Error *a*)	2	0.0903	0.0311
Dates	8	1.9553**	0.8564**
Dates×Blocks (Error *b*)	16	0.0905*	0.0537 n.s.
Dates×Treatments	8	0.0482 n.s.	0.0155 n.s.
Dates×Treat.×Blocks (Error *c*)	16	0.0277	0.0384

Treatment	Means of Eggs Number	Means of Eggs Log of Numbers	Means of Mites Number	Means of Mites Log of Numbers
1. 0.25 pound stabilizer	78.26	1.983	31.94	1.504
2. 1 pound stabilizer	92.60	1.967	47.17	1.674

[a] n.s. not significant, * significant at 5% level, ** significant at 1% level.

Results and Discussion.—The egg and mite data were transformed to log $(x+1)$ and subjected to analyses of variance applicable to a split-block design with treatments in randomized complete blocks (table 4). Although the average number of eggs differed by 14.3 and the adults by 15.3 per sample in the two treatments, these differences were not significant. It was concluded that increasing the amount of stabilizer from 0.25 to 1 pound per 100 gallons did not improve the effectiveness of the 145 formulation in controlling European red mites. It is interesting to note that more mites and eggs were recorded with the 1-pound than with the 0.25-pound treatment. Both treatments had more mites than the comparable treatment of Experiment I (treatment 4), suggesting possibly that the stabilizer may tie up the parathion or interfere with its deposition.

EXPERIMENT III.—*Materials and Methods.*—A third experiment was conducted in a block of 20-year-old McIntosh trees located in the Cornell Orchard, Ithaca, New York, to evaluate the effectiveness of three formulations of demeton[6] in controlling European red mite. Fifteen trees were selected on the basis of uniformity of mite population and uniformity of tree shape and foliage. The three treatments were assigned to the trees to form a completely random design with five replicates. The treatments were applied at the rate of 0.5 pint per 100 gallons with a dilute hydraulic sprayer operated at 300 p.s.i. using a single gun. The sprays were applied from the ground until runoff was observed from all parts of the tree.

Ten leaves were selected from the periphery of each tree at shoulder height and placed in half-pint ice cream cartons. The number of mites (all motile forms) and eggs was determined with a binocular microscope. Samples of 10 leaves each were taken from each of the 15 trees immediately before, and 2, 7, and 14 days after application.

Results and Discussion.—The total number of eggs and motile forms in each sample was recorded and the data transformed to log $(x+1)$. This transformation reduced Bartlett's chi square for homogeneity of variances from 68.73 to 19.45 (chi square 5% level, 11 d.f., 19.68), and

[5] Stabilizer furnished by Diamond Black Leaf Chemical Corp. Composition of the stabilizer is not known.

[6] Three experimental formulations of demeton supplied by Chemagro Corporation.

Table 5.—Analyses of variance of transformed European red mite data, Experiment III.

Source of Variation	d.f.	Mean Square: Eggs Plus Mites	F[a]
Treatments	2	0.0203	0.25 n.s.
Dates	3	6.7981	83.31**
Treatment×Dates	6	0.0097	0.12 n.s.
Error	48	0.0816	

Treatment	Mean—Eggs Plus Mites: Number	Log of Numbers
1. Formulation A	202.8	2.307
2. Formulation B	192.0	2.283
3. Formulation C	175.5	2.244

[a] n.s. not significant, ** significant at 1% level.

removed the proportional relationship between the means and the standard deviations. Analysis of variance applicable to a split-block with treatments in a completely random design was then conducted on these transformed data (table 5). The three treatment means, which differed by approximately 18%, were not significantly different from one another, the large F value obtained in testing the Dates mean square was caused by the rapid and uniform decline of the populations on all trees following the spray application. The lack of a significant Treatment × Dates mean square was probably owing to the equal effectiveness of the three formulations of demeton and to the uniform reduction of the mite populations on all trees.

The results of these experiments demonstrate a method of handling count data from European red mite control experiments with standard statistical procedures. In two of the experiments, the F values failed to reach significance although the treatment differences were sizable. To show significance between treatments in these experiments the error mean squares used to test Treatments would need to be reduced. The error mean square (Treatments×Blocks) may be reduced by (a) increasing the number of blocks in the experiment, (b) increasing the number of sampling dates or (c) increasing the number of leaves sampled. By increasing the number of blocks the degrees of freedom associated with Error *a*, *b* and *c* (Treatment × Blocks, Dates × Blocks, and Dates × Treatments × Blocks, respectively) mean squares would be increased. By increasing the number of sampling dates the precision of estimating the mite population on a tree during the season as well as the degrees of freedom associated with Error *b* and *c* mean squares, would be increased. By increasing the number of leaves at any one sampling date (subsamples) the precision of estimating the mite population on a tree will be improved, as well as decreasing the sampling error mean square. However, increasing the number of subsamples will have little effect in reducing the size of Error *a* or Error *b* mean squares because the degrees of freedom are not increased. Therefore the greatest gain in precision of detecting treatment differences will be obtained by increasing the number of trees sampled.

To demonstrate the effect of increasing the number of blocks on the treatment differences needed for significances, L.S.D.'s were computed using Error *a* (Treatment × Blocks) with 7 treatments, 9 sampling dates and 3, 4, and 5 blocks. These calculations showed that the log treatment means would need to differ by 12.3%, 10.7% and 9.7% in the number of eggs, and 15.4%, 13.6%, and 12.3% in the number of mites in order to be significant when 3, 4, or 5 blocks were used.

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Dimetilan Baits, Fly Ribbons and Cords for the Control of House Flies[1]

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ABSTRACT

Dimetilan® (3-methyl-5-pyrazolyl dimethylcarbamate) incorporated in sugar baits was effective against organophosphorus-resistant house flies (*Musca domestica* L.) in laboratory tests. In field tests in dairy barns in central Florida, against flies known to be resistant to certain organophosphorus insecticides, 1% Dimetilan baits gave effective control in one of two tests. Ribbons impregnated with a sweet syrup and Dimetilan, and cords impregnated with a mixture of parathion and Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate) gave practical control of house flies in hog parlors at Stoneville, Miss., but not in barns and/or poultry houses at Lincoln, Nebr., Kerrville, Tex., and Orlando, Fla. Piperonyl butoxide gave 2½-fold synergism with Dimetilan at a ratio of 10:1 in contact sprays.

Since in many areas house flies (*Musca domestica* L.) have become resistant to some of the organophosphorus insecticides, other groups of compounds have been investigated as possible toxicants. One of the most promising has been the carbamic acid derivatives. At the Orlando, Fla., laboratory 51 carbamates were tested as bait toxicants against malathion-resistant and regular flies. Dimetilan® (3-methyl-5-pyrazolyl dimethylcarbamate) indicated the most promise. This material has been marketed abroad in syrup-impregnated ribbons, a supply of which, together with the technical insecticide, was kindly made available to us by the manufacturer. This paper presents the results of laboratory and field tests at Orlando with Dimetilan in sugar baits, compared with Dipterex® (dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate) and malathion baits as standards, and field tests at Lincoln, Nebr., Kerrville, Tex., Stoneville, Miss., and Orlando with the impregnated ribbons, compared with cords treated with a mixture of parathion and Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate) during 1958 and 1959. Laboratory tests were also made at Orlando to evaluate piperonyl butoxide as a synergist for Dimetilan in contact sprays.

BAITS.—In the laboratory tests acetone solutions of the toxicants at various concentrations were mixed with dry sugar. After drying overnight, 10 grams of each bait was placed in a half-section of a petri dish, and a small cylindrical cage containing 20 female flies was placed on the bait. The flies were allowed to feed *ad libitum*, the knockdown being recorded after 1, 2, and 4 hours and the mortality after 24 hours. At the end of 4 hours a pad of absorbent cotton moistened with water was placed on the cage. The results of quadruplicate tests are summarized in table 1. Dimetilan was superior to malathion in both knockdown and mortality against regular flies, and was almost as effective against malathion-resistant flies as against regular flies.

Field tests with dry sugar baits were run in unscreened dairy barns infested with flies known to have a moderate to high resistance to organophosphorus insecticides. Dipterex was used as the standard. The baits were sifted lightly from a shaker-top jar on floors and entrances of feed and milking rooms where the flies congregated. From 100 to 200 grams were used per building, depending upon the area treated.

Treatments were made daily from Monday through Friday, for 1 week in one series of tests and for 3 weeks in a second series. The infestations were measured by counting the flies that landed in 2 minutes on cloth strips (1×24 inches) saturated with a 50% malt solution. Counts were made each day before the baits were applied

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Table 1.—Toxicity of malathion and Dimetilan baits to malathion-resistant and regular house flies—laboratory tests.

INSECTICIDE AND CONCENTRATION (%)	PER CENT KNOCKDOWN IN			KILL IN 24 HOURS (%)
	1 Hour	2 Hours	4 Hours	
Malathion-Resistant Colony				
Malathion				
0.005	0	0	2	0
.01	0	0	0	0
.025	0	2	2	3
.05	0	0	0	6
.1	2	2	2	2
Dimetilan				
0.005	0	2	5	15
.01	7	9	26	45
.025	9	18	52	91
.05	22	43	78	97
.1	13	29	64	100
Regular Colony				
Malathion				
0.005	0	0	2	12
.01	0	4	14	46
.025	14	17	37	83
.05	24	35	78	100
.1	27	56	89	99
Dimetilan				
0.005	5	18	29	55
.01	15	39	56	78
.025	37	54	80	98
.05	63	92	98	100
.1	67	85	95	95

and again after 10 minutes and 24 hours. The breeding areas on the premises were never treated.

The results are given in table 2. In series 1 the fly density ranged from 68 to 230 per cloth strip in the pretreatment counts. All baits were about equal in effectiveness after 10 minutes; 0.5% Dimetilan was equal to 1% Dipterex, and 1% Dimetilan was superior, after 24 hours. In series 2 the pretreatment counts ranged from 48 to 94 flies per cloth strip. Again the Dimetilan formulations were equal or superior to Dipterex in the 10-minute counts, but effective control was obtained only in one of the two barns treated with 1% Dimetilan bait.

RIBBONS AND CORDS.—Dimetilan ribbons and commercial fly cords were tested in barns and other farm buildings. The ribbons were 2 meters long and composed of a spongy material impregnated with a sweet syrup and 1 gram of Dimetilan per meter. The fly cords were of cotton, containing 13.79% of parathion and 3.54% of Diazinon. Both were installed below the ceiling in accordance with the manufacturers' directions—one ribbon or 30 feet of cord per 100 square feet of floor area, the ribbons 10 cm. and the cords 1 foot below beams or rafters. The fly populations before and after treatment were measured by means of the malt-saturated cloth strips described in the preceding section, except at Kerrville, Tex., where they were made by means of Scudder fly grills. At several locations it was found that the ribbons disintegrated on aging.

TESTS AT LINCOLN, NEBR.—Ribbons were installed in four buildings, and cords in three. The sizes and types of the buildings are given in table 3. The ribbons were installed a few inches below the ceilings; the cords were installed 12 inches below 8-foot ceilings in two buildings, and in a third, a Quonset hut, they were stretched horizontally 7 feet from the floor, or about 17 feet below the maximum height of the curved roof.

The results are given in table 4. Control with the Dimetilan ribbons was somewhat better than that with

Table 2.—Per cent reduction of house flies in dairy barns after treatment with sugar baits containing Dimetilan and Dipterex, Orlando, Fla.

DAYS AFTER FIRST TREATMENT	DIMETILAN 0.5%		DIMETILAN 1%				DIPTEREX 1%			
			Test 1		Test 2		Test 1		Test 2	
	10 Min.	24 Hours	10 Min.	24 Hours	10 Min.	24 Hours	10 Min.	24 Hours	10 Min.	24 Hours
Series 1										
0	8	—	5	—	3	—	41	—	32	—
1	26	62	75	96	46	39	43	67	26	0
2	18	68	50	98	55	52	62	76	53	0
3	32	0	40	95	64	68	44	81	37	0
4	26	92	50	98	67	91	33	0	—	—
Series 2										
0	74	—	80	—	82	—	60	—	—	—
1	93	42	35	14	60	82	48	56	—	—
2	71	55	72	0	50	88	35	31	—	—
3	75	46	70	43	0	89	58	25	—	—
5	58	0	73	52	67	95	56	13	—	—
8	84	—	78	—	66	—	63	—	—	—
9	59	11	64	9	83	51	40	63	—	—
10	75	29	72	38	60	92	57	22	—	—
11	63	33	60	95	100	97	80	64	—	—
12	55	0	63	0	100	98	60	0	—	—
15	83	—	—[a]	—[a]	63	—	80	—	—	—
16	38	16	—	—	82	98	38	0	—	—
17	70	0	—	—	100	92	46	73	—	—
18	36	0	—	—	50	96	61	32	—	—
19	56	18	—	—	100	92	51	73	—	—

[a] Treated by owner, test discontinued.

Table 3.—Characteristics of buildings treated with ribbons and cords for house fly control at Lincoln, Nebr.

Treatment and Location	Size of Barn (feet)	Height of Ceiling (feet)	Remarks
Ribbons			
Damrow	42×50	8	Hog parlor. Cattle feed lot next to building. Breeding pressure moderate.
Hoy	18×56	7–9	Shed, frequented by hogs and cattle, attached to barn used to store feed and machinery.
	20×32	8	Hog parlor located 20 yards from building. Breeding pressure moderate to heavy.
Polenz	16×18	8	Milking parlor.
	10×18	8	Calf stalls. A loafing shed, 26×40 feet, was not treated, few flies frequented it. Breeding pressure moderate.
Skyline	24×30	8	Calf barn ¼ mile from dairy. Breeding pressure moderate.
Cords			
Allen	30×36	8	Milking and calf barn combined. Breeding pressure light to moderate.
Myers	25×40	8	Hog parlor. Beef cattle were being fattened in nearby lot. Breeding pressure moderate.
University	42×60	0–24	Quonset hut housing feed bunks for beef cattle. Many flies in partially shaded lot outside of hut.

the parathion-Diazinon cords, but was not satisfactory with either. Although the ribbons appeared to be attractive to the flies and the insecticide was highly toxic, the poor control was apparently caused by their relative inaccessibility while the flies were feeding. In the barns with low ceilings the flies did not abandon the ceiling, beams, electrical cords, and so forth, in preference to the fly cords as resting surfaces at night. At the Quonset hut some flies rested at night high on the ceiling and others stayed in trees a few feet from the hut.

The Poultry Husbandry Department, University of Nebraska, installed fly cords in a poultry house for the control of house flies. The building was about 1,300 square feet in area and had a slatted floor under which the manure accumulated. It had only three openings—one door and two high-speed air-intake fans on one side. It was unlikely that flies could enter freely except when the door was opened for men to enter or leave. Approximately 1,200 laying hens were kept in the building. The cords were placed below the 7½-foot-high ceiling at a rate almost twice that recommended. The fly population was suppressed for a few days, but within 4 weeks there was an extremely high population, and it was evident that the cords were failing to give a practical degree of control.

Table 4.—Per cent control of house flies at Lincoln, Nebr., with Dimetilan ribbons and parathion-Diazinon cords, 1959.

Time After Treatment	Ribbons				Cords		
	Damrow (300)[a]	Hoy (29)	Polenz (52)	Skyline (95)	Allen (60)	Myers (37)	University (45)
Days							
1	—	59	65	94	0	46	0
2	95	66	—	—	28	—	0
3	—	90	—	90	28	—	0
4	96	—	—	98	—	40	—
Weeks							
1	86	0	73	63	0	76	0
2	85	76	89	66	0	0	4
3	94	14	82	79	—	21	0
4	75	0	82	85	—	0	—
5	56	0	80	70	—	—	—
6	—	—	94	37	—	—	—
7	—	—	37	78	—	—	—
8	—	—	—	33	—	—	—

[a] Numbers in parentheses indicate pretreatment counts.

Table 5.—Characteristics of buildings treated with ribbons and cords for house fly control at Kerrville, Texas.

Treatment and Location	Size of Barn (feet)	Height of Ceiling (feet)	Remarks
Ribbons			
Heinen milk barn	27×20	11	On rafters 7 feet above floor.
Parker dairy			
Milk barn	29×17	8	On rafters 7 feet above floor.
Bull barn	62×18	10	On rafters 7 feet above floor.
Cords			
Pfeiffer poultry house	92×22	13	On rafters 7 feet 10 inches high. Building also contained electrocuting device consisting of four rows of paired wires.
Cords and ribbons			
Le Meilleur dairy			
Milk barn	30×11	9	On rafters 6 feet high.
Calf barn	20×10	7	Cords and ribbons tied 4 from roof.

Table 6.—Per cent control of house flies at Kerrville, Tex., with Dimetilan ribbons and parathion-Diazinon cords, 1959.

Time After Treatment	Heinen Dairy		Parker Dairy		Le Meilleur Dairy				Pfeiffer Poultry House,
					Milk Barn		Calf Barn		
	Milk Barn, Ribbons (33)[a]	Calf Barn, Check (25)	Milk Barn, Ribbons (17)	Bull Barn, Ribbons (28)	Cords (12)	Ribbons (9)	Cords (36)	Ribbons (21)	Cords (7)
Days									
1	30	—	73	36	42	0	31	0	0
3	60	—	28	64	30	0	16	24	35
Weeks									
1	92	—	9	36	27	0	43	58	5
2	95	45	34	60	—	33	—	—	—
3	80	19	77	81	—	—	—	—	—
4	80	3	52	52	—	—	—	—	—
5	95	7	40	43	—	—	—	—	—
6	91	2	—	—	—	—	—	—	—
7	49	16	—	—	—	—	—	—	—

[a] Numbers in parentheses indicate pretreatment count.

TESTS AT KERRVILLE, TEX.—Ribbons were installed in five barns, and cords in two barns and one poultry house. The characteristics of the buildings are given in table 5, and the results in table 6. Fairly good control was obtained only at the Heinen milk barn, where ribbons were used. The ribbons reduced the populations to some extent at the other locations, but flies were still too abundant for control to be considered satisfactory. The cords gave such small reductions that the cooperators were forced to abandon the tests. The owner of the poultry house treated it with a residual spray and the test was terminated. At the Le Meilleur dairy a nonresidual space spray was used, and the cords were removed and replaced with ribbons.

TESTS AT STONEVILLE, MISS.—Cords were installed in two hog parlors, and ribbons in four. The characteristics of the premises are shown in table 7. All situations, except Taylor's, were cotton plantations having hog and cattle operations as secondary projects. Each parlor had a concrete slab floor that was ordinarily washed once a day. Cords and ribbons were attached below and at right angles to the run of joists or rafters except that in the Atterbury house twenty 3-foot cords were attached to the rafters in areas of fly concentrations and allowed to hang perpendicularly beginning on the 12th day.

Reasonably high control was obtained in all parlors but flies were not eliminated completely in any location and, in fact, flies were considered of nuisance level when control was below 92% at Atterbury, 94% at Taylor, 85% at Collier No. 1, 65% at Collier No. 2, 85% at Ainsworth and 94% at Alexander. The results are presented in table 8.

TESTS AT ORLANDO, FLA.—Cords were installed in two dairy barns, and ribbons in three. The characteristics of the barns are given in table 9. Since house flies in Florida barns tend to congregate on or near the floor, in two barns the cords and ribbons were mounted on wooden racks at the rate of 40 feet of cord or 3 feet of ribbon per rack. The racks were 40 inches long, and elevated the cords or ribbons 6 inches from the floor. The results are given in table 10.

None of the treatments gave satisfactory control; reductions caused by the racks were as high as or higher than those caused by the suspended cords or ribbons, although much smaller quantities of cords or ribbons were

Table 7.—Characteristics of buildings treated with cords and ribbons for house fly control at Stoneville, Miss.

Treatment and Location	Size of Barn (feet)	Height of Ceiling (feet)	Remarks
Cords			
Atterbury	20×40	4–20	15–18 brood sows. Lean-to roof attached to hay barn; half of area in farrow pens, half for feeding slaughter pigs. Sanitation good, drainage inadequate.
Taylor	20×40	8	Centrally located among other farm buildings. Sanitation fair, wastes flushed into sluggish bayou.
Ribbons			
Collier No. 1	20×36	8	60 brood sows on pasture; 80–100 slaughter pigs in parlor. Parlor separate from farrowing units. Sanitation good, waste drained into bayou.
Collier No. 2	24×32	8	Closed farrowing building except for doors at ends.
Ainsworth	20×30	5½–10	35–50 pigs in parlor; also 15–20 sows. Isolated building by 200 yards, on concrete slab. Sanitation fair, drains into open ditch.
Alexander	60×24	14	20–25 sows on pasture; 200 feeder pigs in parlor. Concrete slab near other farm buildings. Sanitation fair; waste drained into open ditch, eventually into irrigation system.

Table 8.—Per cent control of house flies at Stoneville, Miss., with Dimetilan ribbons and parathion-Diazinon cords, 1959.

Days After Treatment	Cords		Ribbons			
	Atterbury (352)[a]	Taylor (459)	Collier #1 (161)	Collier #2 (73)	Ainsworth (176)	Alexander (402)
3	90	73	91	85	82	88
5	90	91	93	90	92	95
7	—	93	92	89	—	—
10	86	96	95	92	94	97
15	86	97	96	95	95	98
20	87	95	96	96	97	99
30	91	98	96	92	97	99
40	95	98	98	96	95	93
50	95	98	98	95	97	97
60	89	98	95	89	95	93
75	78	98	95	90	97	97
90	—[b]	96	75	80	90	74
105	—	94	72	69	89	49
120	—	89	70	89	96	—

[a] Numbers in parentheses indicate pretreatment count.
[b] Sprayed for another purpose.

used. However, it was necessary to treat each barn with bait occasionally when flies became too numerous for the owner to tolerate.

Synergist Tests.—Wind-tunnel tests with contact sprays were made to evaluate piperonyl butoxide as a synergist for Dimetilan at a 10:1 ratio. Female house flies from the regular colony were exposed momentarily to fine sprays of the insecticide in odorless kerosene, as the spray was drawn through a wind tunnel containing the caged flies. After treatment the flies were moved to clean cages and observed for knockdown and mortality. The results are given in table 11. Approximately 2½-fold synergism was exhibited by the combination.

Table 9.—Characteristics of dairy barns treated with cords and ribbons for house fly control at Orlando, Fla.

Treatment and Location	Size of Barn (feet)	Height of Ceiling (feet)	Remarks
Cords			
Stevens	72×33	7–15	Strung below 7-foot ceiling in feed room and below 8-foot rafters in milking room. Tests in 1958 and 1959.
Ribbons			
Hiatt	115×24	10–15	Suspended from rafters 10 feet high.
Brockbank	100×34	8–15	Suspended from 8-foot ceiling in milking area, and from 10-foot rafters in feed room.
Cords on racks			
Farmer	75×36	10–15	Four racks on floor.
Ribbons on racks			
Judge	102×40	10–15	Four racks on floor.

Table 10.—Per cent control of house flies at Orlando, Fla., with Dimetilan ribbons and parathion-Diazinon cords.

Days After Treatment	Ribbons		Cords (Stevens)		Ribbons on Racks (Judge) (172)	Cords on Racks (Farmer) (197)
	Hiatt (90)[a]	Brock-bank (208)	1958 (234)	1959 (212)		
1	11	78	0	45	92	47
2	42	79	—	—	57	0
3	0	22	18	—	—	—
4	0	—	—	62	—	—
5	—	—	—	73	81	48
6	—	74	—	76	50	0
7	0[b]	50	13	37	68	31
8	0	93[c]	—	68[b]	78	0
9	0	95[c]	0	—	75[b]	0[b]
10	33	97	—	—	—	—
11	31[b]	—	—	—	—	—
12	—	—	—	63	81	92
13	—	94	—	—	—	—
14	0	—	0	57[b]	63	91
15	—	64	—	—	—	—
16	0	—	—	—	69[b]	87[b]
17	—	75[b]	—	—	—	—
18	0	—	—	—	—	—
19	—	—	—	43	96	91
20	—	83	—	—	—	—
21	0	—	0	0	85	84
22	—	76	—	—	—	—
23	0[b]	—	—	—	—	—
24	—	—	—	—	—	—
25	—	—	—	—	—	—
26	—	—	—	0	96	92
27	—	66	—	—	—	—
28	53	—	—	0	81	97
29	—	85	—	—	—	—
30	0	—	—	—	87	93

[a] Numbers in parentheses indicate pretreatment count.
[b] Treated with 1% Dipterex bait to reduce fly population.
[c] Heavy rains in area sharply reduced fly population.

Table 11.—Effectiveness of Dimetilan, alone and synergized with piperonyl butoxide (1:10), in wind-tunnel tests against regular-colony house flies.[a]

Concentration (%)		Knockdown (%)			Kill in 24 Hours (%)
Dimetilan	Piperonyl Butoxide	10 Min.	30 Min.	60 Min.	
2.5	0	100	100	100	100
1.0	0	83	100	100	95
0.5	0	85	100	100	93
.25	0	18	70	80	23
.1	0	3	58	85	18
.05	0	0	3	5	5
2.5	25.0	100	100	100	100
1.0	10.0	98	100	100	100
0.5	5.0	73	100	100	98
.25	2.5	33	100	100	98
.1	1.0	5	43	73	53
.05	0.5	3	5	13	15
0	10.0	0	0	0	0
0	5.0	0	0	0	3
0	2.5	0	0	0	0
0	1.0	0	0	0	0
0	0.5	0	0	0	0
0	.25	0	0	0	3
0	.1	0	0	0	0
Check	—	0	0	0	3

[a] Average of 2 tests of 20 females each.

Control of the Snake Mite (*Ophionyssus natricis*), Other Mites, and Certain Insects with the Sorptive Dust, SG 67[1]

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Abstract

A highly porous, finely divided, amorphous type of silica aerogel, SG 67, with an average particle size of 3 micra was found to be very effective for the control of the snake mite, *Ophionyssus natricis* (Gervais), infesting snakes and lizards, and the cages housing them. The compound kills by adsorbing the lipids (waxes) making up the waterproof layer of the epicuticle of arthropods causing the loss of abnormal quantities of body fluids in comparatively short periods of time. The dust compound is composed of 95.3% silica aerogel with 4.7% ammonium fluosilicate impregnated in less than a continuous monomolecular film on its internal surfaces. The ammonium fluosilicate gives the compound a strong positive charge enabling it to adhere well to the bodies of mites as well as to the horizontal and vertical surfaces of the construction materials used for the reptile cages and to the particles of sand and vermiculite used to cover the floors of the cages. The ammonium fluosilicate greatly increases the insecticidal effectiveness of the dust compound, but does not detract from the ability of the silica aerogel particles to adsorb the lipids from the epicuticle. A gopher snake placed into 10 ounces of SG 67 for a period of 1 week, a ring-neck snake submerged in approximately 1 ounce of the compound for more than 18 hours, and an alligator lizard thoroughly doused with the dust suffered no ill effects from the compound. More than 100 snakes and lizards now have been successfully treated with the compound for the control of snake mites and none has been affected adversely by the compound. A single dusting with 7 ounces of SG 67 per reptile cage, 6 feet long by 3 feet wide and 1½ feet high, on the inside framework and the vermiculite and sand flooring of the cage controlled the mites within the cage, as well as on reptiles housed in the cage, for a period of 16 months. Without exception, merely placing the reptiles in the cages and allowing them to crawl over the treated surfaces of the cages was sufficient to accomplish rapid control of the mites on the reptiles.

The snake mite, *Ophionyssus natricis* (Gervais), has long been recognized as a troublesome, blood-sucking parasite of snakes and lizards in zoological parks and herpetological laboratories. Snakes and lizards heavily infested with this mite become listless and emaciated, refuse to eat, and die or become useless for exhibition or experimental work. According to Camin (1953), adult female mites ingest large enough quantities of blood during a single blood meal to increase their weight 15-fold. Several hundred mites feeding on even a large snake can produce severe anemia. *Ophionyssus natricis* was found by Camin (1948) to be a mechanical vector of *Proteus hydrophilus*, the causative organism of a severe and often fatal hemorrhagic septicemia in snakes. Noninfected snakes placed in cages with infected snakes became infected only if the mites also were present.

A number of acaricides are toxic to snakes and lizards and cannot be used. Pyrethrum and sulfur powders and Sulphenone® (*p*-chlorophenyl phenyl sulfone) dusts have been recommended as safe and effective acaricides for snakes and lizards, but herpetologists recently have reported getting unsatisfactory mite control with them and toxic reactions in reptiles ingesting them.

It has long been known that chemically inactive dusts of fine particle size can kill arthropods by adsorbing or abrading the protective lipid layer of the epicuticle. When this layer is so damaged, death due to resultant abnormal water losses ensues.

Chiu (1939a) found bentonite the most effective of six inert materials he tested for killing adult bean weevils (*Acanthoscelides obtectus* (Say)). Later Chiu (1939b) found crystalline silica the most effective compound for killing rice weevils (*Sitophilus oryza* (L.)) and granary weevils (*S. granarius* (L.)). After examining 400 different dusts, Alexander *et al.* (1944) found hard materials such as diamond, carborundum and quartz, effective in killing grain weevils (*Calandria granaria* L.), soft substances, such as talc, china clay and gypsum, ineffective, and compounds of intermediate hardness, such as fluorite and glass, only moderately effective. They also noted that dusts have to adhere well to an insect's body in order to be effective and that the finer the particle size the greater the covering power and adhesion of a dust. Particles of carborundum above 15 micra had no effect on the insects, but effectiveness increased as the particle size was reduced from 10 micra to about 2 micra. Of 61 inert diluents tested on the Mexican bean beetle (*Epilachna varivestis* Muls.), Hunt (1947) found Barden Clay the most effective and reported that desiccation followed abrasion with the clay. Of various silica compounds tested on adults of the confused flour beetle (*Tribolium confusum* Duv.), the rice weevil, the granary weevil, and larvae of the confused flour beetle and the Mediterranean flour moth (*Anagasta kühniella* (Zell.)), Cotton & Frankenfeld (1949) found a silica aerogel the most effective. Heavy treatments of grain with this compound produced 100% mortality of insects in 1 to 2 weeks, whereas marginal dosages required 5 or 6 weeks to give complete kill. They concluded that light applications of the silica aerogel to the surface of wheat in farm storage bins might help prevent infestations by incoming grain insects, but didn't recommend use of the compound throughout grain as it then would be classified as "treated," graded as "limed," and thereby reduced in market value. In studies to determine the

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effectiveness of various dusts on worker honey bees, Jones (1955) found the most sorptive dusts most effective in desiccating the bees. He also found materials such as silica gel, Almicide and activated charcoal with a particle size small enough to pass through a 300-mesh sieve more effective than coarser materials. Nair (1957) found that the nature of the outermost layer of the epicuticle influences the adherence of dusts in that more clings to insects covered with soft wax than with hard wax or cement.

Past attempts to control arthropods with desiccating dusts principally have been directed against granary insects. In studies to determine the effectiveness of inert dusts on drywood termites (*Kalotermes minor* Hagen), Wagner & Ebeling (1959) screened 162 compounds and found sorptive dusts more effective than abrasive materials, and silica gels, silica aerogels and silica precipitates the most effective. The silica aerogels are now being used commercially for the prevention of drywood termites in home attics.

In laboratory studies carried out by the writer to determine the effectiveness of various inert dusts on household pests and on arthropods affecting man and animal, the more than 110 test compounds included natural, synthetic, precipitated, fluoride-treated and coated silicas; synthetic calcium and aluminum silicates; diatomaceous earths; montmorillonite, attapulgite, bentonite and kaolinite clays; cement drifts and activated carbons. A synthetic silica aerogel, SG 67, used industrially as a flatting agent for lacquers and as an antiblocking agent for inks and paper coatings, was found singularly effective. This compound is a highly porous, finely divided, amorphous type of silica aerogel. It is odorless, white, has an average particle size of 3 micra, a surface area of 300 square meters per gram, and when packed has a density of 4.5 pounds per cubic foot. One pound of the compound will adsorb 2.55 pounds of oil. The compound is composed of 95.3% silica aerogel with 4.7% ammonium fluosilicate impregnated in less than a continuous monomolecular film on the internal surfaces of the compound. The ammonium fluosilicate, originally added for industrial purposes completely unrelated to arthropod control, gives the compound a strong positive charge that enables it to adhere exceptionally well to the bodies of arthropods, reptiles, birds and mammals and to vertical and horizontal surfaces of almost all building and construction material. The ammonium fluosilicate greatly increases the insecticidal effectiveness of the compound, without affecting the adsorptive properties of the silica aerogel particles.

Rapid death of many arthropods owing to desiccation, initiated by adsorption of the lipid layer by SG 67, has been accomplished experimentally by the writer with German (*Blattella germanica* (L.)), American (*Periplaneta americana* (L.)), oriental (*Blatta orientalis* L.) and brown-banded (*Supella supellectilium* (Serv.)) cockroaches; cat (*Ctenocephalides felis* (Bouché)) and oriental rat (*Xenopsylla cheopis* (Rothsch.)) fleas; brown dog (*Rhipicephalus sanguineus* (Latr.)) and spinose ear (*Otobius megnini* (Dugés)) ticks; bedbugs (*Cimex lectularius* L.); the short-nosed cattle louse (*Haematopinus eurysternus* (Nitz.)); house flies (*Musca domestica* L.); mosquitoes (*Culex tarsalis* Coq.); conenose bugs (*Triatoma protracta* (Uhler)) and field crickets (*Acheta assimilis* F.).

The compound has been extensively field tested for the control of dog and cat fleas (Tarshis 1959a); German, oriental and brown-banded cockroaches (Tarshis 1959b, 1959c); brown dog ticks (Tarshis & Dunn 1959); short-nosed cattle, wild bird, rodent and walrus lice; bed bugs; wild bird and tropical rat mites; dermestid and pantry beetles; Argentine ants and silverfish.

LABORATORY STUDIES.—*Preliminary Experiments with Mites Treated with SG 67.*—During laboratory studies to determine the effectiveness of SG 67 on a variety of arthropods, preliminary experiments also were carried out with this compound on the following species of mites (*Haemolaelaps glasgowi* (Ewing), *Dermanyssus gallinae* (DeG.), *Pimeliaphilus podapolipophagus* Tragardh, *Ornithonyssus bacoti* (Hirst) and *O. sylviarum* (Canestrini and Franzago). The mites for these studies were obtained from various laboratories and held until needed in holding jars in an incubator maintained at 80° F. with an approximate relative humidity of 50%. Five test groups of 50 to 100 mites, one group of each species in varying stages of development, were selected from the holding jars and placed into 1-inch diameter stender dishes, each of which contained 20 mgs. of the test compound. A second series of five groups, each containing the same number of mites of each species, as in the first groups, were placed into 1-inch stender dishes but without the test compound and were used as checks. The tops of all the stender dishes were well greased with a mixture of petroleum jelly and glycerine to prevent the escape of the mites during the test period.

At an ambient temperature of 77° F. with an ambient humidity of 56% the immature mites in the test compound of each group were knocked down within 15 minutes and the adult mites in 30 minutes. The immature mites were dead within 20 minutes and the mature mites within 60 minutes. Microscopic observations made on the mites shortly after exposure to the test compound showed their bodies beginning to become depressed, particularly their abdomens, indicating the loss of copious quantities of body fluids. Twenty-four hours following the initiation of these experiments, all the mites in the checks were still alive.

Experiments with Mites of the Genus Tyrophagus.—Following the initial trials with SG 67 on the above-mentioned species of mites, the work had to be discontinued owing to a lack of mites with which to work. However, a small colony of *Tyrophagus* mites was collected from the debris of a culture of German cockroaches in the writer's laboratory and an attempt was made to colonize these mites in sufficient numbers for test purposes. The colony of mites in varying stages of development was placed into a wide-mouth gallon jar containing approximately 250 grams of pulverized Purina Dog Chow Checkers. A plastic container, 2½ inches high and 3 inches in diameter, containing a water-soaked pad of absorbent cotton, was placed into the culture jar to increase the humidity. The 50% humidity in the temperature cabinet to be used for rearing the mites was not sufficiently high for them and could not be raised without harming other arthropods in the temperature cabinet. *Tyrophagus* mites in all stages of development died in 30 to 42 hours at a humidity of 50%. To prevent the escape of the mites, the top 2 inches inside the jar was well-greased with a mixture

of petroleum jelly and glycerine and the top of the jar was covered with a screw cap centered with 100 mesh bronze strainer cloth. The culture was incubated at 80° F. The mites reproduced and thrived so well in the culture media and culture jar that within a period of 1 month, thousands of mites were available and all laboratory experiments reported on in this paper were done with mites of the genus *Tyrophagus*.

Exposure of Tyrophagus Mites to SG 67 Held at Different Relative Humidities.—This experiment consisted of two parts (1) the exposure to SG 67 of mites removed from the culture media and held at four different relative humidities and (2) exposure to SG 67 of mites contained in culture media and held at four different relative humidities. The second part of this experiment was done to determine just how effective the test compound would be on mites moving about in the culture media and able to hide under the particles to avoid any of the dust; also, to find out how well the compound would adhere to the culture particles held at four different relative humidities.

For part one of the experiment, eight groups of several hundred mites each, in varying stages of development, were removed from the culture media. Each of four groups of the mites was placed into a 2-inch diameter stender dish containing a 50-mg. sample of SG 67. Each of the remaining four groups of mites was placed into a clean stender dish and was used as a check.

For part two of the experiment, eight 5-ml. samples of culture media, each containing several hundred mites in varying stages of development, were selected at random from the culture jar and each was put into one of eight 2-inch diameter stender dishes. A 50-mg. sample of SG 67 was put into each of four of the dishes and, with an applicator stick, was thoroughly mixed with the mites and culture media in the dish. Four of the stender dishes of mites and culture remained untreated and were used as checks. The tops of all 16 stender dishes were coated with a mixture of petroleum jelly and glycerine to prevent the escape of mites.

Each test group of four stender dishes, 1 check with mites only, 1 check with culture media and mites, 1 with mites treated with SG 67 and 1 with culture media and mites treated with SG 67, was placed into each of four humidity chambers; one held at 25% humidity, one at 50%, one at 75% and one at 100%. The stender dishes were removed from the humidifying chambers at 3-hour intervals up to 12 hours, at 6-hour intervals up to 48 hours and at 24-hour intervals up to 120 hours when the experiment was terminated.

The mites in all the stender dishes containing SG 67 were dead at the end of 3 hours at all humidities. Of the untreated mites used as checks, all immature stages were dead after 30 hours and all adults after 42 hours at the 25% and 50% humidities. All stages of the untreated mites held at 75% or 100% humidities remained alive for more than the 120 hours the experiment was run. The results of this experiment are summarized in table 1. The dead, dust-treated mites were well desiccated at all humidities. The test compound was found adhering to all surfaces of the particles of the culture media, as well as to the mites' bodies.

Weight Losses of Tyrophagus Mites Untreated and Treated with SG 67.—In this experiment the weights of 2 groups of 8 plastic weighing bottles (2 replicates of each), 2 inches high by 1-inch in diameter, and covers for each were recorded. The covers consisted of small pieces of Silverlight membrane secured to the weighing bottles with tight fitting plastic rings.

Sixteen groups of several hundred mites each, in varying stages of development, were removed from the culture media and each group was placed into a weighing bottle, and the bottle again weighed to determine the weight of each group of mites. A 25-mg. sample of SG 67 was placed into each of eight weighing bottles and the remaining eight bottles of mites were left untreated and used as checks.

Each test group of 4 weighing bottles, 2 with mites treated with SG 67 and 2 with untreated mites, was placed into one of the four humidity chambers; one was held at 25%, one at 50%, one at 75% and one at 100%. The test chambers were removed from the humidifying chambers at the end of 3 hours, 6 hours and 24 hours and weighed to determine losses in weight of the mites. The ambient temperature during the experiment was between 77° and 80° F.

The mites treated with SG 67 lost the greatest percentage of weight after 3 hours at 25% humidity, lost no additional weight by the end of 6 hours and lost a slight amount more by the end of 24 hours at this humidity (table 2). The treated mites held at 50% humidity also lost the greatest percentage of weight after 3 hours, with appreciably smaller percentages of loss in weight after 6 and 24 hours. However, there was an appreciable difference in losses in weight of the mites held at 75% humidity.

Table 1.—Hours required for 100% mortality of immature and mature *Tyrophagus* mites exposed to SG 67 and held at four different humidities and an ambient temperature of 77° to 80° F.

Humidity															
25%				50%				75%				100%			
Checks		Treated		Checks		Treated		Checks		Treated		Checks		Treated	
Im.[a]	M.[b]	Im.	M.	Im.	M.	Im.	M.	Im.	M.	Im.	M.	Im.	M.	Im.	M.
Mites Out of Culture Medium															
30	42	3	3	30	42	3	3	—[c]	—[c]	3	3	—[c]	—[c]	3	3
Mites in Culture Medium															
30	42	3	3	30	42	3	3	—[c]	—[c]	3	3	—[c]	—[c]	3	3

[a] Immature stages of mites.
[b] Mature mites.
[c] Mites still living after 120 hours.

Table 2.—Weight losses of *Tyrophagus* mites treated with SG 67 and held for 3, 6 and 24 hours at 25%, 50%, 75% and 100% humidities and at an ambient temperature between 79° and 82° F.

Relative Humidity (%)	Per Cent Loss in Weight After Hours Shown					
	3		6		24	
	Check	Treated	Check	Treated	Check	Treated
25	23.3	51.5	30.0	51.5	41.7	53.5
50	20.4	41.5	25.9	42.3	35.2	48.1
75	3.8	17.1	5.1	20.1	9.0	22.0
100	0.0	0.0	0.0	0.0	0.0	0.0

The difference in recorded losses between treated mites held at 25% and 50% humidities and those held at 75% humidity is attributable to the fact that at ambient temperatures of 76° to 80° F. and 25% and 50% humidities SG 67 does not absorb any moisture, but at 75% humidity the compound does absorb some moisture.

Though the percentages of losses in weight were recorded as zero for mites treated with SG 67 and held at 100% humidity the mites actually did lose weight, for the mites were dead at the end of 3 hours and when examined with a stereoscopic microscope were observed to be just as well desiccated as those that died at 25% and 50% humidities. The gain in weight of the dust at 100% humidity, through moisture absorbed (table 3), made it impossible to record losses in weight for the mites.

The untreated checks showed the greatest losses in weight at the end of 3 hours at 25% and 50% humidities, with appreciably smaller percentages of losses in weight after 6 and 24 hours. At 75% humidity the untreated checks lost very little weight and at 100% humidity the untreated checks lost no weight. The percentages of moisture absorbed by SG 67 at four different humidities for varying periods of time are shown in table 3.

The Effect of SG 67 on the Eggs of Tyrophagus Mites and Some Insects.—In this experiment several hundred mite eggs were removed from the culture media and a group of approximately 50 eggs was placed into each of six 1-inch diameter stender dishes, 3 containing 20 mg. of SG 67 and 3, used as checks, without SG 67. One check dish and one dish containing mites and test compound were placed into 75% and 100% humidifying chambers. The remaining two dishes were kept at room temperature (75° to 80° F.) with an ambient humidity of 46% to 54%. Weekly examinations were made of the eggs for a period of 6 weeks. The eggs were not held at 25% and 50% humidities owing to the fact that at these humidities immature and adult *Tyrophagus* mites die in 30 to 42 hours, whereas these stages can be kept alive at the higher humidities.

SG 67 was found to have no effect on the eggs of these mites. Almost all of the dead larvae in the test chambers were completely free of the eggs and completely desiccated. A few completely desiccated larvae were found halfway emerged from the eggs and a few eggs were unhatched, but did not appear at all desiccated. In the untreated dishes almost all of the larvae emerged from the eggs, but a few did remain unhatched.

Similar studies made by the writer on eggs of *C. felis* and *X. cheopis* showed that SG 67 also had little effect on the eggs of these fleas. Again, a number of larvae were found halfway out of the egg shell and desiccated, the rest of the larvae were free from the egg shell and completely desiccated in the dust compound. The oöthecal sacs of the German, American, oriental and brown-banded cockroaches exposed to SG 67 were not affected by the compound, but once the nymphs emerged they were killed rapidly by the compound. Dying or dead gravid females of these four species of cockroaches exposed to the compound over a period of several weeks were found to still contain oöthecal sacs that were as completely desiccated as the adult cockroaches were.

In another study when the hair on the tails of Holstein cattle heavily infested with eggs of *H. eurysternus* was dusted with SG 67, or the hair clipped from the tail and then dusted, the nymphs that emerged from the eggs and came in contact with the dust died very quickly. However, a number of eggs that were still covered with the dust were found to have nymphs halfway emerged from the eggs and completely desiccated. When opened, the dusted unhatched eggs were found to contain completely desiccated, fully mature embryos. Several egg masses of the brown dog tick dusted with SG 67 became completely desiccated after exposure in the compound for approximately 35 days, at temperatures between 70° and 76° F. and humidities between 35% and 56%, although a few eggs did hatch. The emerged ticks died from contacting the dust. All the ticks emerged from untreated egg masses in the same period of time held at the same temperatures and humidities.

Field Studies.—Field evaluation studies with SG 67 for the control of *O. natricis* infesting snakes and lizards were carried out during the latter part of 1958 at the herpetological laboratories in the Department of Zoology at the University of California at Los Angeles. The mites were so numerous on the snakes and lizards that they were seen protruding from beneath the folds of the skin and scales, around the eyes and freely moving about on the skin surfaces.

Though finely powdered sulfur had been used to treat the infested reptiles and the laboratory cages housing them, the mite infestations had not been controlled and, in some instances, the dust had an adverse effect on the treated reptiles.

Preliminary SG 67 Tests on Snakes and Lizards.—In order to determine whether SG 67 could be used to control *O. natricis* on reptiles, a heavily mite-infested gopher snake, *Pituophis catenifer annectens*, was placed into a 64-ounce receptacle containing approximately 10 ounces of SG 67. The snake was left in the dust for a period of a week, then removed and examined for mites and to

Table 3.—Per cent moisture absorbed by SG 67 held at 25%, 50%, 75% and 100% relative humidities and an ambient temperature between 76° and 80° F. in varying periods of time.

Relative Humidity (%)	Per Cent Moisture Absorbed in Varying Periods of Time (hours)				
	3	6	24	48	72
25	0	0	0	0	0
50	0	0	0	0	0
75	0.3	0.4	0.5	0.5	0.5
100	2.6	4.5	13.1	18.5	24.5

determine whether the compound had any adverse effects on the snake. The snake was found to be free of mites and suffering no ill effects from its long exposure to the dust. Additionally, a heavily mite-infested alligator lizard, *Gerrhonotus multicarinatus*, rather than being placed into the dust compound as the gopher snake had been, was thoroughly "bathed" with the dust and then placed into a clean container. Within several hours no live mites were to be found and the dust remaining on the lizard produced no ill effects. The snake and lizard were exposed to the dust at a temperature of 77° F. with a relative humidity of 44%. In a later test, a mite-free ring-neck snake, *Diadophis amabilis* (perhaps more susceptible to drying than many reptiles), suffered no ill effects from SG 67 after it had been submerged in the compound (1 ounce) for more than 18 hours at a temperature of 70° F. with a relative humidity of 50%.

Application of SG 67 for Snake Mite Control.—Since the preliminary trials showed that SG 67 could control mites on a gopher snake and an alligator lizard without producing ill effects, each of four infested reptile cages was dusted with the compound. The cages were 6 feet long by 3 feet wide and 1½ feet high, made of glass and angle iron, and covered at the top with screen cloth. The floors of the cages were made of wood and covered with a mixture of vermiculite and sand. The lizard and snake cages were heated with heat lamps and temperatures were held around 80° F. The relative humidity was between 42% and 46%.

Two ounces of the dust were applied with a hand duster to all inside metal surfaces, including cage frames, lighting fixtures and heating elements in each cage. It is important that this be done thoroughly since the female mite, upon completion of her blood meal, will leave the host to deposit her eggs in cracks and crevices or on rough metal surfaces. An additional 5 ounces of the dust was strewn over the surface of the vermiculite and sand mixture covering the floor of each cage. When applying the dust it is not essential to work it into the vermiculite and sand or other material used on the floor of reptile cages as the movement of the reptiles over the floor will do this. However, working the dust in while applying it does give more rapid initial control of the mites, and eliminates any unsightly appearance. The reptiles themselves need not be treated; they will pick up enough of the dust while crawling over the treated floor covering to control any mites present on their bodies. However, if mites hide out under the chin shields and in the eye sockets and if they become particularly abundant in these areas it might be advisable to apply the dust directly to these areas.

During the past 16 months, from the time of the sole dusting, the reptile cages and the experimentally-treated gopher snake and alligator lizard have remained free of mites. Though none of the reptiles that were exposed to the dust compound were examined microscopically for the detection of mites, at no time during the period the reptiles were in the treated cages were they observed to have mites crawling over their bodies. During the 16-month period, 50 mite-infested snakes (gophers; sidewinders; Pacific, red and speckled rattlers; racers; garters) and 50 mite-infested lizards (alligators Northern cresteds, fringe foots, chuckwallas, desert iguanas) have been housed in these treated cages for varying periods of time. Without exception, the mites on these infested reptiles have been brought rapidly under control by merely placing the reptiles in the treated cages.

Table 4.—Recovery of snake mites from the sand flooring of a gopher snake cage before and after dusting with SG 67.

No. of 5-Ml. Samples of Sand Examined	Mites Recovered			
	Alive Before Treatment[a]		Dead After Treatment[b]	
	Larvae	Nymphs and Adults	Larvae	Nymphs and Adults
1	41	5	18	17
2	34	1	22	8
3	38	4	28	18
4	30	3	16	12
5	38	5	15	10
6	28	16	18	5
7	27	8	35	6
8	16	8	19	12
9	10	9	17	5
10	15	6	10	5
11	20	3	12	7
12	25	15	19	14
13	28	6	30	11
14	26	8	35	15
15	32	10	41	12

[a] No dead mites recovered.
[b] No living mites recovered.

On April 14, 1960 another mite-infested gopher snake and cage were treated with SG 67. The cage, made of ½-inch plywood, measured 22½ inches in length, 10 inches high and 9 inches wide and had a 1½ inch layer of medium coarse white sand covering the floor. Many adult mites were observed crawling over the snake's body, but the greatest concentration of mites was found in the sand on the floor of the cage. In order to determine just how heavy the infestation was in the sand, sand samples totaling 75 ml. were taken at random. The sand was divided into 15, 5-ml. samples and each 5-ml. sample was redivided into 1-ml. samples and placed into petri dishes for careful examination with a stereoscopic microscope for the presence of live mites. Each sand sample was found to be well-infested with live mites in varying stages of development.

With a hand duster the inside wooden sides of the cage were well-dusted with 1/8-ounce of SG-67. Particular care was exercised in placing the dust at all joints and in cracks and crevices. The surface of the sand was dusted with 1 ounce of SG 67 and worked into the sand with a tongue depressor. The snake was not removed from the cage while the inside of the cage was being dusted. The snake had been and continued to be kept in a laboratory where the temperature was 77° F. with a relative humidity of 44%.

The following morning, 22 hours after the cage was dusted, the snake was found to be in good condition, suffering no ill effects from the dusting and was free of mites. The snake was covered with a fine film of dust which did not detract from its appearance.

Again, sand samples totaling 75 ml. were taken from the floor of the cage at random and examined as described above. No live mites were found. The dead larval mites were well covered with the test compound and were so

completely desiccated that they were difficult to detect. The dead nymphs and adults also were well covered with the test compound, and though completely desiccated, could be distinguished from the sand particles and debris in the sand. All surfaces of each sand particle and piece of debris were well covered with the dust compound. The tabulations on the number of live mites found in the sand on the cage floor prior to dusting and the number of dead mites in the sand 22 hours after the cage was dusted are given in table 4. Sand samples taken from the cage at 2-week intervals for a period of 2½ months and examined with a stereoscopic microscope also were found to be free of live mites.

In the latter part of April, 1960, experimental work with SG 67 was started at the San Diego Zoo on the control of ectoparasites of zoo animals. A number of reptile cages and reptiles, including Gaboon Vipers (*Bitis gabonica gabonica*), corn snakes (*Elaphe guttata guttata*) and black rat snakes (*Elaphe obsoleta obsoleta*) have been treated with SG 67 for the control of snake mite infestations. To date the compound has controlled the mite infestations on these snakes and in the cages, and has had no adverse effects on the snakes.

The silica aerogel, SG 67, is manufactured by the Davison Chemical Division of the W. R. Grace Company, Baltimore, Maryland. The compound can be obtained under the trade name Dri-Die 67 from pet shops, pesticide suppliers and distributors.

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Studies on Fine-Particle, Coarse-Particle, and Concentrated Aerosols[1]

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ABSTRACT

Aerosols of fine particle size (2 to 5 microns m.m.d.) were found to be about as good as those of coarse particle size (15 to 20 microns m.m.d.). The important factor was the concentration of insecticide dispersed per unit volume of air. The nonvolatile content of an aerosol formulation is not of much importance as long as the spray droplets do not exceed the aerosol range.

On this basis, concentrated aerosol formulations were prepared containing up to 10% of active ingredient (allethrin) with no other adjuvants and these were found to be highly effective on nonresistant and resistant house flies.

In order to approach the maximum efficiency of aerosols in insect control, considerable effort has been expended in finding the optimum particle size and other physical characteristics as well as suitable insecticidal formulations. Two major types of liquefied gas-propelled sprays have been developed—(1) the conventional aerosol, which produces particles usually in the range of 15 to 20 microns mass median diameter (m.m.d.), ordinarily used for control of flying insects; and (2) a coarse, wet type with droplets of 50 microns m.m.d. or higher, used to put down a residual film for control of crawling insects. Sprays of the latter type are not true aerosols, which have been defined as having 80% or more of the particles below 30 microns and none larger than 50 microns (Fulton & Rohwer 1949).

The studies described herein were aimed at improving aerosols to be used on aircraft. However, since the same principles can be applied for use in rooms, most of the tests were carried out in rooms of 1,000 cubic feet or larger, rather than in Peet-Grady chambers (216 cubic feet), in order to approach the volumes of air space encountered in aircraft.

Two objectives in aircraft disinsectization are (1) to secure good dispersal of the insecticide so that insects in all parts of the cabin and other compartments will be killed, and (2) to obtain a high concentration of insecticide in the air for a long enough time to obtain a high or complete kill. These objectives would seem to be most readily attained by gaseous or volatile insecticides—in

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other words by fumigation, but unfortunately there are no volatile insecticides available that are safe enough for use in the presence of people, although some attempts have been made to use lindane in this manner (Sullivan 1952, Quarterman & Sullivan 1953). The next best line of attack would be to utilize a safe insecticide in such finely divided form that the resulting aerosol would approach the fumigating action of a gas. However, there is a practical limit to this concept, since the finer the particles are the higher will be their leakage rate through cracks, crevices, windows, or other openings. Fine particles are distributed better than coarse particles by thermal or other air currents in rooms or planes, they impinge less on air-conditioning ducts and fans, and, even more important, they stay suspended in air much longer. We therefore chose to work in the range of 2 to 5 microns m.m.d. for fine-particle tests because it was felt that smaller particles would impinge less readily on insects and would have too high a leakage rate under normal conditions of use.

During these studies it was found that aerosol formulations could be prepared containing an insecticide of high purity and a propellent with no other volatile or nonvolatile adjuvants. Therefore some tests were made of concentrated formulations.

MATERIALS TESTED.—

I. Allethrin, 0.5%; propellent 11, 49.75%; propellent 12, 49.75%; 2 microns m.m.d.

II. Allethrin, 0.5%; deodorized kerosene, 14%; propellent 11, 42.75%; propellent 12, 42.75%; 20 microns m.m.d.

III. Pyrethrins, 0.5% (2.5% of a 20% concentrate); propellent 11, 48.75%; propellent 12, 48.75%; 7 microns m.m.d.

IV. Allethrin, 0.5%; DDT, 0.1%; propellent 11, 49.7%; propellent 12, 49.7%; 2 microms m.m.d.

V. DDT, 2.0%; allethrin, 0.6%; methylene chloride, 4.0%; Freon 11, 46.7%; Freon 12, 46.7%; 10 microns m.m.d.

VI. Military-type formulation.—DDT, 2.0%; allethrin, 0.6%; alkylated naphthalene, 5.0%; deodorized kerosene, 7.2%; Freon 11, 42.6%; Freon 12, 42.6%; 20 microns m.m.d.

VII. 1% Allethrin formulation.—Allethrin (93.6% pure), 3.2 gm.; propellents 11 and 12 (50:50), 296.8 gm.; 5 microns m.m.d.

VIII. 10% Allethrin formulation.—Allethrin (93.6% pure), 10.6 gm.; propellents 11 and 12 (50:50), 89.4 gm.; 20 microns m.m.d. P-2, 6-ounce cans were used.

IX. Pyrethrins, 7%.—Pyrethrins (70% experimental concentrate), 10.0 gm.; propellents 11 and 12 (50:50), 90.0 gm.; 20 microns m.m.d.

X. Coarse-particle, concentrated (4×) military aerosol.—DDT, 8 gm.; allethrin (93.3% pure), 2.6 gm.; methylene chloride, 20 gm.; cyclohexanone, 10 gm.; propellent 12, 59.4 gm.; 25 microns m.m.d.

XI. Coarse-particle, slow-delivery, 10% allethrin formulation.—Allethrin (90% pure), 11.1 gm.; propellent 12, 88.9 gm.; 20 microns m.m.d. An army-type can equipped with a Dill army-type valve modified with a 0.010-inch I.D. capillary inserted into the orifice in the valve cap was used, and the delivery rate was 0.61 gm. per second.

Unless otherwise specified, a 12-ounce seamless Crown can and a green Precision valve with a 0.0175-inch diameter orifice was used.

In all experiments house flies (*Musca domestica* L.) 2 to 3 days old National Association of Insecticide and Disinfectant Manufacturers (NAIDM) 1948 nonresistant strain, were used unless otherwise stated.

EXPERIMENT 1.—The first experiment was carried out to compare the effectiveness of 2-micron and 20-micron m.m.d. aerosols containing allethrin as the toxicant (formulations I and II).

The particle-size range of these two aerosols by the waved-slide method of Yeomans (1949) was as follows:

2 microns m.m.d.		*20 microns m.m.d.*	
Microns Diameter	*Accumulative Per Cent*	*Microns Diameter*	*Accumulative Per Cent*
2	50	5	0.5
4	90	10	5
6	94	15	20
8	97	20	50
10	100	25	57
		30	72
		35	80
		40	85
		45	98
		50	100

The aerosols were released about 6 feet above the floor while the operator was moving in a circular pattern around the room. Windows were shut and taped to minimize leakage. About 200 flies were released in each room. Dosages of 42 ± 4 mg. of allethrin per 1,000 cu. ft. were checked by weighing the aerosol cans before and after discharge. Treatments were made at 3 p.m., and mortality counts after the flies had been exposed for 18 hours. Small containers of sugar water were placed in the rooms during the tests. The rooms used were 1,152 and 1,536 cubic feet.

The results of seven tests are given in table 1. In two tests the temperature dropped so low that the mortality exceeded 90% because of the negative temperature coefficient of toxicity for allethrin. The results show that, in tightly closed rooms with an 18-hour exposure, 2-micron m.m.d. allethrin aerosols are slightly more effective than

Table 1.—Effectiveness of allethrin aerosols of 2 and 20 microns m.m.d. particle size (formulations I and II, experiment 1) against house flies.[a]

PER CENT MORTALITY		TEMPERATURE (°F.)	RELATIVE HUMIDITY (%)
Formulation I 2 Microns m.m.d.	Formulation II 20 Microns m.m.d.		
47	33	99–84	37–48
82	59	97–80	55–49
93	74	76–69	68–52
97	92	75–66	52–54
68	53	96–82	55–49
84	78	92–80	50–32
75	80	93–70	24–36
Average 78	67	90–76	49–46

[a] Dosage, 42 mg. of allethrin per 1,000 cu. ft.; exposure time, 18 hours.

Table 2.—Effectiveness of 2-micron m.m.d. allethrin and pyrethrins aerosols against house flies (experiment 2).[a]

Formulation	Insecticide and Dosage (mg./1,000 cu. ft.)	Temperature (°F.)	Mortality in 24 Hours (%)
	1,824-cubic foot room		
I	Allethrin 540	84	100
	270	100	96
	135	79	98
		77	100
		80	97
		92	99
		Av. 82	Av. 98.5
III	Pyrethrins 135	78	33
		78	47
		81	26
		83	45
		Av. 80	Av. 37.8
	1,150-cubic foot room		
I	Allethrin 100	81	77
IV	Allethrin 100 plus DDT 20	78	98
I	Allethrin 68	73	39
		79	71
		81	93
		80	81
		Av. 78	Av. 71
IV	Allethrin 68 plus DDT 13.6	77	79
		76	77
		78	96
		81	98
		Av. 78	Av. 87

[a] About 200 free-flying house flies exposed for 1 hour.

20-micron m.m.d. aerosols. Observations of knockdown also showed the former aerosols to be somewhat better than the latter.

EXPERIMENT 2.—This experiment was carried out to compare fine-particle aerosols of 2 to 7 microns m.m.d. containing allethrin (I), pyrethrins (III), and allethrin plus DDT (IV) against free-flying house flies. The exposures were reduced to 1 hour. Tests with formulation IV were made to determine the effect of incorporating a small amount of DDT along with allethrin without the use of a cosolvent.

The results given in table 2 indicate that allethrin is superior to pyrethrins against house flies. The addition of only 0.1% of DDT caused a significant increase in kill.

EXPERIMENT 3.—A fine-particle aerosol of 4 microns m.m.d. containing 0.6% of allethrin plus 2% of DDT and having no nonvolatile cosolvents (formulation V) was compared with a 17-micron m.m.d. military-type aerosol containing the same insecticides and the usual nonvolatile cosolvents (15% total nonvolatile content, formulation VI).

Both nonresistant NAIDM 1948 and resistant LDD-9 Beltsville house flies were used. Caged yellow-fever mosquitoes (*Aedes aegypti* (L.)) were also exposed in two tests. Doors and windows were closed but not taped.

Table 3 shows that the 4-micron m.m.d. aerosol (V) was as effective as the 17-micron military-type (VI) at the same dosages of active ingredients. This, together with results of previous tests, shows that aerosols from 20 microns m.m.d. down to as low as 2 microns m.m.d. in particle size, and formulations with nonvolatile content from the conventional 15% to as low as 0.5%, give very similar results when tested in rooms at the same concentrations of active ingredients in the air with exposures of ½ hour or more.

In some of the tests air samples were taken with an electrostatic sampler which draws 3 cubic feet of air per minute through the sampling tube. All the aerosol particles drawn into the apparatus were precipitated out on the inside of a metal tube. The tube was then washed out with absolute ethanol and the solutions analyzed by ultraviolet absorption spectroscopy with a Beckman DK-2 spectrophotometer using the base line technique. Analytical calibration curves were prepared using DDT plus allethrin in the same ratio as used in the aerosols (10:3). The tubes containing samples of aerosols from the military-type formulation (VI) were allowed to stand in air overnight before being washed out with ethanol in order to allow the methylated naphthalene and refined kerosene to evaporate, and thus minimize interferences from these substances.

The results of these analyses are given in table 4. Although theoretically more of the 4-micron than of the 17-micron m.m.d. aerosol should be left in the air after 1 hour, the differences found by analyses were not as great as might be expected, where the sampler was placed in one corner of the room 6 feet from the floor. However, it is possible that the higher leakage rate of the fine aerosol through cracks around doors and windows offset the advantage of its slower settling rate.

EXPERIMENT 4.—After it was found that nonvolatile matter of little or no insecticidal effectiveness could be eliminated from aerosol formulations, it was decided to

Table 3.—Room tests (1,150–3,240 cubic feet) to determine the relative effectiveness of 4- and 17-micron m.m.d. allethrin aerosols (formulations V and VI) against normal and resistant house flies (experiment 3).

Test Number	Formulation	Particle Size (microns m.m.d.)	Dosage Used (g./1,000 cu. ft.)	Temperature (° F.)	Mortality in 24 Hours (%)
		NAIDM Nonresistant House Flies			
1a	V	4	3.3	71	100
1b			3.0	72	97
1c			2.3	74	100
			Av. 3.0	Av. 72	Av. 99
2a	VI	17	1.7	69	98
2b			3.0	73	98
2c			2.2	72	93
			Av. 2.3	Av. 71	Av. 96
3	V	4	2.8	82	100
4	VI	17	2.8	82	100
5	V	4	1	70	89[a]
6	VI	17	1	70	79[b]
		LDD-9: Beltsville Resistant House Flies			
7a	V	4	2.8	72	41
7b			2.8	70	39
			Av. 2.8	Av. 71	Av. 40
8a	VI	17	2.8	74	42
8b			2.8	70	21
			Av. 2.8	Av. 72	Av. 32

[a] 100% mortality was obtained for caged mosquitoes located at various heights in the room.

[b] 80% mortality was obtained for caged mosquitoes located at same stations used in test No. 5.

Table 4.—Chemical analyses of amounts of insecticide in the air during tests with aerosol formulations V and VI (experiment 3).

Test Numbers[a]	Sampling Time (minutes)	Particle Size (microns m.m.d.)	Room Size (cu. ft.)	Dosage (mg./1,000 cu. ft.) Theoretical		Insecticide Found (mg./1,000 cu. ft.)		Per Cent of Theoretical
				DDT	Allethrin	DDT	Allethrin	
Sampler near center of room 3 feet from floor								
1b (A)	2	4	1,150	61	18	67	20	110
1b (B)	10					11	3	19
1c (A)	4	4	1,150	47	14	41	12	87
1c (B)	10					7	2	16
3 (A)	4	4	3,240	56	17	30	9	53
3 (B)	15					10	3	18
4 (A)	4	17	3,240	56	17	28	8	49
4 (B)	15					3	1	5
Sampler in corner of room 6 feet from floor								
7b (A)	15	4	3,240	57	17	16	5	28
7b (B)	20					4	1	7
8b (A)	15	17	3,240	57	17	13	4	23
8b (B)	20					3	1	5

[a] The test numbers correspond to those in table 3. (A) refers to collection of sample as soon after release of the aerosol as practicable. (B) refers to collection of sample just prior to the end of the 1-hour test.

try a formulation in which the nonvolatile content was made up entirely of a high-purity insecticide (90% or higher) at a concentration that would produce particles of the conventional aerosol size (15 to 20 microns m.m.d.). Accordingly, a formulation (VIII) containing 10% of allethrin and 90% of propellents (20 microns m.m.d.) was compared with one containing 1% of allethrin and 99% of propellents (5 microns m.m.d., formulation VII).

These tests were run in a brick building with a gambrel roof, with numerous skylights on the roof and large windows on the southern exposure. A large room in the building, 97 feet 7 inches long, 24 feet 3 inches wide, and 10 feet 8 inches high (average height) contained about 25,600 cubic feet of space. Silicone-coated microscope slides were placed on the floor throughout the room. Caged yellow-fever mosquitoes of the Orlando strain were placed at five equally-spaced stations along the room. All doors and windows were closed and about 500 three- to six-day-old house flies were then released. The fine- and coarse-particle allethrin aerosols (VII and VIII) were released at a dosage of 1.14 grams of allethrin (44.5 mg. per 1000 cu. ft.). Applications were made from the center of the room with the bomb directed upward and outward while the operator slowly pivoted on the central spot rather than having the operator walk up and down or using a fan in the room in order to emphasize any effects owing to differences in distribution.

The insects were exposed for 30 minutes and the knocked-down flies were collected, fed, and placed in cages for 24 hours. The flies not knocked-down were estimated visually and the number added to the number of flies which had recovered after the 24-hour observation period. The microscope slides were collected 25 minutes after the start of the test. The results of these tests are given in table 5.

A study of the microscope slides showed that a small number of particles 30 microns in diameter were present in both the fine and coarse aerosols. These particles did not travel more than 15 feet from the point of spraying. The few 30-micron diameter particles from the fine aerosol were caused by some frost formation on the nozzle due to the long release time (a metal nozzle would eliminate this trouble). No particles larger than 12 microns were found on the slides placed at the ends of the room. There was not sufficient air movement owing to convection currents to distribute the aerosols evenly throughout the closed room during the 30-minute exposure. Therefore, in a room of this size when applications were made as in these tests, best results might be obtained if the aerosol contained no particles larger than about 12 microns or if it was released as the dispenser was carried the length of the room.

Table 5.—Effectiveness of fine- and coarse-particle aerosols (formulations VII and VIII) against house flies in a 25,600-cubic foot room; ½ hour exposure period; 44.5 mg. of allethrin per 1,000 cubic feet (experiment 4).

Temperature (°F.)	Knockdown in 30 Minutes (%)	Mortality in 24 Hours (%)
	Formulation VII	
66–67	99	61
73–74	97	76
74–75	98	78
79	85	54
81–84	98	59
	Av. 95.4	Av. 65.6
	Formulation VIII	
66–67	92	47
73–77	93	84
70–73	76	51
82	61	29
81–86	86	48
	Av. 81.6	Av. 51.8

Table 6.—Comparative effectiveness of the military aerosol bomb (formulation VI) with one four times as concentrated (formulation X) against house flies in a 10,000-cu. ft. room; 500 female flies per test (experiment 6).[a]

Spraying Time (seconds)	Knockdown After ½ Hour (%)		Mortality of Knocked-down Flies in 24 Hours (%)	
	Test 1	Test 2	Test 1	Test 2
	Military Aerosol VI			
70	98	89	100	100
35	71	78	99	100
20	44	58	95	94
10	38	42	97	92
	Concentrated (4×) Military Aerosol X			
18	84	80	100	100
9	81	69	100	100
5	86	64	99	99
2.5	74	68	95	93

[a] Acknowledgment is made to the Orlando, Florida laboratory of the Entomology Research Division for the data in this table.

The kill of house flies was better and the knockdown considerably better for the fine- than for the coarse-particle aerosols. The kill of mosquitoes was 100% with both aerosols.

EXPERIMENT 5.—Since experiment 2 indicated that in fine-particle aerosols allethrin was considerably more effective against house flies than pyrethrins, it seemed desirable to obtain data on the relative effectiveness of these insecticides in aerosols of conventional particle size (15 to 20 microns m.m.d.) when generated from concentrated formulations in the absence of other toxicants, synergists, or cosolvents. In order to formulate a concentrated pyrethrins aerosol, the ordinary 20% pyrethrins concentrate obviously could not be used, since a 10% pyrethrins formulation would require 50% of a 20% concentrate. We therefore used a 7% pyrethrins aerosol (IX) containing 10 grams of an experimental 70% pyrethrins-concentrate and 90 grams of propellents 11 and 12 (50:50).

This aerosol was compared with a 10% allethrin aerosol (VIII) in 1000-cubic feet chambers and replicated seven times. The results, presented in figure 1, show that allethrin was about twice as effective as pyrethrins.

In tests at the Orlando, Florida, laboratory of the Entomology Research Division, the regular military aerosol formulation (VI) was compared with an allethrin-DDT formulation that was four times as concentrated (X). Five hundred regular Orlando colony female flies were released in a room of 10,000 cubic feet. After the flies had been allowed to orient for ½ hour, aerosols were discharged for given periods of time—VI for 70, 35, 20, and 10 seconds and X for periods only one-fourth as long to give equivalent dosages of active ingredients. One-half hour later the knocked-down flies were picked up, placed in a cage, and held for 24-hour mortality counts. The results given in table 6 show that the concentrated 4× military aerosol (X) was equal or superior to the regular military formulation (VI) except on the knockdown obtained at the longest spraying times.

EXPERIMENT 6.—Resistance of flying insects to DDT is rather widespread and is becoming an increasingly vexatious problem. Most aerosol formulations contain the comparatively expensive allethrin or pyrethrins in very

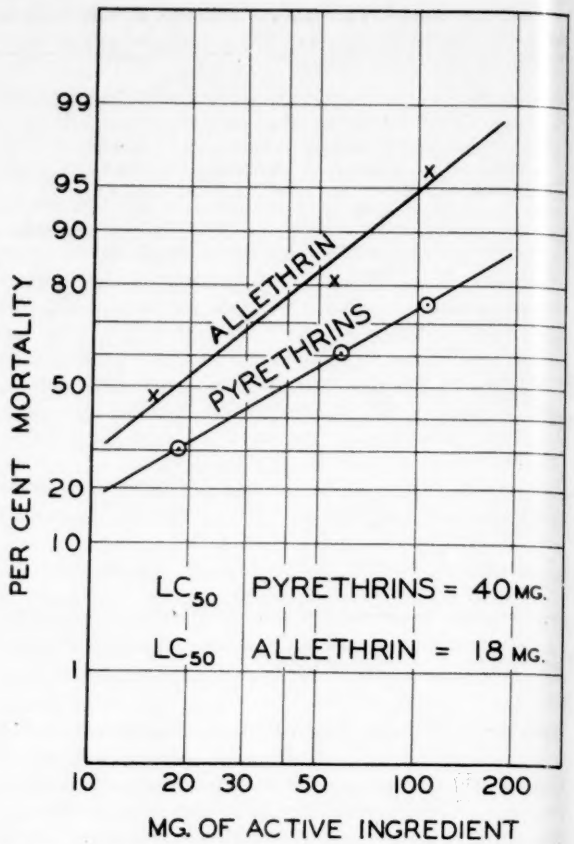

FIG. 1.—Tests of a 10% allethrin aerosol (VIII) and a 7% pyrethrins aerosol (IX) in 1000-cu.-ft. chambers, seven replications.

low concentrations mainly for their knockdown action and depend for killing power on an insecticide of little knockdown activity, such as DDT or methoxychlor. Against many resistant strains such formulations would be rather ineffective. The advantage of increasing the content of an insecticide, such as allethrin, to which insects do not show high resistance, is demonstrated in table 7, which gives results of a comparison of a 10% allethrin formulation (VIII) with an allethrin-DDT formulation of the military type (VI) against Orlando-Beltsville resistant house flies in 1000-cubic-feet chambers. The 10% allethrin aerosol was considerably more effective against the resistant house flies than the military formulation when compared on the basis of total active ingredients.

Table 7.—Comparison of a 10% allethrin bomb (VIII) with an allethrin-DDT military formulation (VI) against Orlando-Beltsville resistant house flies in 1000-cu. ft. chambers (experiment 6).

10% Allethrin Aerosol		Military Aerosol (DDT + allethrin)		
Allethrin (mg.)	Mortality (%)	DDT (mg.)	Allethrin (mg.)	Mortality (%)
42	10	16	5	2
332	57	190	57	8
490	95	362	109	41

Table 8.—**Effectiveness of a 10% allethrin aerosol formulation (XI) with a slow delivery rate (0.61 gram per second) against resistant house flies (experiment 6).**[a]

Dosage of Allethrin (mg./1,000 cu. ft.)	Knockdown		Mortality in 24 Hours (%)
	First Insect Down (min.)	Per Cent ½ Hour	
Orlando-Beltsville Resistant House Flies			
100	4	94	60
	4	98	46
200	4	100	74
	2	100	85
300	3	100	91
	3	100	87
Fales 1958 Resistant Wild Fly Strain			
300	1	100	99
	1	100	99

[a] Insects exposed for ½ hour in 2,400 cu. ft. rooms.

Where it is desired to decrease the delivery rate, a smaller orifice in the valve cap may be used. Table 8 shows that satisfactory results may be obtained with the slow delivery rate used with formulation XI.

DISCUSSION.—From the experimental work, these basic conclusions may be drawn:

(1) The important factor in the toxicity of aerosols to flying insects is the concentration of active ingredients dispersed per unit volume of air; fine-particle aerosols as low as 2 to 5 microns m.m.d. are as good as 15 to 20 microns m.m.d. coarse-particle aerosols under the conditions used in our tests.

(2) The per cent of nonvolatile matter in formulations is of little consequence as long as the spray droplets do not exceed the aerosol range; therefore, it is not necessary to use cosolvents or noninsecticidal nonvolatile oils unless they are required in the formulation.

There is a mass of evidence, both theoretical and practical (Latta *et al.* 1947, LaMer *et al.* 1947, LaMer & Hochberg 1949, Johnstone *et al.* 1949, Nelson *et al.* 1949, Yeomans *et al.* 1949, Fulton 1951, Yeomans 1952, Fales *et al.* 1956) pointing to the superiority of aerosols of 10 to 20 microns m.m.d. for the control of flying insects and to the rapid fall in effectiveness as the particle size decreases below 10 microns. In addition, the optimum nonvolatile content was generally considered to be 10% to 15%. We have no explanation for the apparent differences from the present work, except that many of the earlier tests were carried out for shorter exposure periods, which limited the settling time, and in small enclosures, which limited the free flight of the insects.

The Effect of Particle Size on Insecticidal Efficiency.—LaMer *et al.* (1947), in their work with DDT-aerosols on mosquitoes in confined atmospheres with exposures of ½ to 30 minutes, concluded that the toxicity as measured by the time of exposure-concentration product is increased 250 or more times by increasing the diameter of the particles from 0.33 to 11 microns. LaMer & Hochberg (1949), in tests of DDT-aerosols on yellow-fever mosquitoes in confined atmospheres and in wind tunnels, concluded that the particle size should be about 10 microns diameter or higher for optimum results. From the work of Latta *et al.* (1947) with DDT-aerosols in wind-tunnel tests on yellow-fever mosquitoes, the optimum particle size appeared to be about 12 microns in a 2 m.p.h. (mile per hour) wind. In a discussion by Johnstone *et al.* (1949) of some theoretical aspects and mathematical calculations relating to the dispersion and deposition of aerosols, the calculated results agreed closely with the experimental observations of LaMer and coworkers. Yeomans *et al.* (1949) calculated that the optimum diameter of aerosol particles for impingement on mosquitoes flying at 2 m.p.h. was 15.81 microns as compared with 22.4 microns for house flies flying at 4 m.p.h. Yeomans (1952) stated that for adult mosquitoes the optimum particle size was between 10 and 15 microns and for adult house flies between 15 and 20 microns. In some warehouse treatments he found that 1 to 5 microns was best for protection of stored products mainly from crawling but also from some flying insects. However, in such cases it was more desirable to obtain as complete and uniform distribution of the insecticide as possible, chiefly in order to get an adequate, even residual deposit, than to obtain any rapid kill of such flying insects as moths.

Fine-particle aerosols (2 to 5 microns m.m.d.) have certain advantages over coarse-particle aerosols (15 to 20 microns m.m.d.). In confined atmospheres where there is little air-exchange or leakage the fine-particle allethrin aerosols seem to be more effective in kill and definitely superior in knockdown. Because the fine particles stay suspended for hours and are distributed better by even slight thermal convection of other air currents, they can remain effective longer. Circulation through air-conditioning ducts and fans without too much impingement might be possible with fine-particle aerosols but not at all with coarse aerosols.

The one disadvantage of fine-particle aerosols seems to be that they require a higher proportion of propellent to dispense the same amount of insecticide. However, future studies may show how to produce them on a small scale more economically (they can be made easily on a large scale by smoke generators and fog machines). Although allethrin is considered safe pharmacologically (Carpenter *et al.* 1950, Starr *et al.* 1950, Ambrose & Robbins 1951), fine-particle allethrin aerosols, which can penetrate deeper into the lungs than conventional aerosols, will require further study from this standpoint.

Concentrated Aerosols.—For the production of conventional, coarse-particle aerosols (15 to 20 microns m.m.d.), concentrated formulations have a number of advantages over the usual formulations that have been used. For military use, where logistics and weight are prime considerations, the high killing power and knockdown obtainable per unit weight of such formulations make possible the use of small, lightweight containers particularly adaptable for the use of individual, forward troops.

Allethrin is especially well suited for concentrated formulations. Any concentration of allethrin up to about 10% or possibly higher, can be used depending on the size or price of the container, number of dosage units per container, type of application, or other desiderata of the formulator. The rationale of employing 10% allethrin is based on the fact that by eliminating other insecticides, synergists, or cosolvents, and by utilizing the maximum amount of allethrin consistent with particle-size limitations (10% of allethrin plus 90% of propellents 11 and

12 gives about 20-micron m.m.d. aerosols), a maximum amount of killing and knockdown power can be obtained with a minimum amount of propellent; this is particularly true when consideration is given to the fact that flying insects are becoming resistant to DDT.

Tests with allethrin formulations up to 20% utilizing the higher pressure obtained with propellent 12 alone are described elsewhere (Sullivan *et al.* 1960).

Effect of Nonvolatile Content on Insecticidal Efficiency.—McGovran *et al.* (1943) and Fales *et al.* (1946) found that about 15% non-volatile content gave the best results in tests of DDT aerosols against house flies in a Peet-Grady chamber. Nelson *et al.* (1949) after studying many formulations concluded that the best formulations appeared to be those containing 10% to 15% of non-volatile constituents and recommended about 15% for formulations. All other factors being equal, the higher the nonvolatile content, the higher will be the mass median diameter of the aerosol particles.

Differences Between Present and Previous Test Methods.—In the present work larger test chambers (1,000 cubic feet or larger) and longer exposures (one-half hour or more) were used than in most previous work. The size of the chamber may affect the flight and resting behavior of flying insects. Also, convection and distribution patterns are likely to be different in different-sized rooms. As to exposure time, most insecticide aerosols (15 to 20 microns m.m.d.) do not settle out in less than one-half hour and the finer ones in less than several hours. Under normal conditions, insects would be exposed either until they flew out of the treated room or until the aerosol was dissipated by settling or leakage. It seems that larger testing chambers (1,000 cubic feet or larger) and longer exposure times (one-half hour or longer) are highly desirable in tests with aerosols.

Another difference is that allethrin and pyrethrins were used as the chief toxicants in most of the present work. They differ from DDT and most other insecticides in having an initial stimulatory action soon followed by a paralytic or knockdown effect even at dosages considerably below those needed to obtain a high kill. These properties are particularly valuable in formulations used to control flying insects.

Allethrin as a Toxicant in Insecticidal Aerosols.—Allethrin is particularly well suited to aerosol formulations. It is readily obtainable in 90% or higher purity; it is miscible with propellents in all proportions; there are no serious corrosion or formulation problems; it has very low mammalian toxicity; it has no staining properties; it has only a slight, mild odor at low concentrations although high concentrations are somewhat irritating, like the pyrethrins; it has both high toxicity and good knockdown action on flying insects; and it is effective on DDT-resistant flies. Pyrethrum presents greater formulation problems in that it has a higher propellent-insoluble content, and more important, it is only available in concentrates containing 20% to 25% of pyrethrins. Therefore, the highest concentration obtainable in an aerosol formulation is about 2% to 3% of pyrethrins unless a more highly concentrated extract can be supplied commercially. One of the most concentrated pyrethrum aerosol formulations sold commercially contains 1.2% of pyrethrins and 6% of piperonyl butoxide.

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Susceptibility of Mosquitoes to Silica Gel Insecticides[1]

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ABSTRACT

Adult female *Anopheles quadrimaculatus* Say, *Culex fatigans* Wied. and susceptible and DDT-resistant *Aedes aegypti* (L.) were exposed for periods of 1 hour to filter paper treated with silica gel insecticides, using the World Health Organization standard test kit for adult mosquitoes. Dosages of a silica aerogel (Dri-Die) as low as 50 mg./sq. ft. produced high mortalities in all species, the effectiveness being somewhat reduced in tests at 100% relative humidity. Susceptible and DDT-resistant strains were equally affected. Aqueous suspensions of this insecticide were less than half as effective as the dust. Death was due in part to desiccation and was frequently accompanied by the formation of exudates appearing as small, spherical or hemispherical masses on the scutum, legs or wings of the mosquitoes. Silikil-D dust and aqueous suspensions at high dosages exhibited little effectiveness against *A. aegypti* although mortality was greater at a high relative humidity.

The effectiveness of silica gels as residual insecticides against certain species of insects has been reviewed recently along with an appraisal of some of the physical and biological factors which appear to influence their insecticidal action (Ebeling & Wagner 1959). Much of the promise which these chemicals show in insect control lies in their apparent lack of toxicity to mammals and their sorptive capacity to which insects and other arthropods may not become resistant.

With the rapidly increasing problem of insecticide resistance in disease vectors, such as mosquitoes (Micks 1960), the necessity of investigating as many different types of insecticidal agents as possible is obvious. It was therefore of particular interest to determine the residual effectiveness of silica gels against adult mosquitoes, both susceptible and insecticide-resistant.

MATERIALS AND METHODS.—Laboratory-reared strains of *Anopheles quadrimaculatus* Say, *Culex fatigans* Wied. and susceptible (University of Texas, Medical Branch) and DDT-resistant (Trinidad) *Aedes aegypti* (L.) provided the test material. Adult females of 3 to 5 days of age and more or less engorged with sugar solution were used for all tests reported herein. The standard World Health Organization (WHO) test kit for adult mosquitoes was employed. Test papers were prepared by distributing weighed quantities of Dri-Die (silica aerogel) and Silikil-D (silica gel) evenly over the filter paper using a camel's-hair brush. One series of papers was prepared by spraying them with aqueous suspensions of Dri-Die with a nasal atomizer and allowing them to dry before use. Dri-Die test papers were treated at the rate of 50, 100, 150 and 200 mg./sq. ft. and the same set of papers was used for all tests with dust. Silikil-D was applied at dosages of 250 and 500 mg./sq. ft.

Twenty to thirty mosquitoes were introduced into each holding tube and observed for at least 30 minutes in order that any traumatized specimens might be replaced. They were then transferred, as in the WHO test procedure, to exposure tubes containing the silica gel-treated papers. Following 1 hour exposure, the specimens were returned to the holding tubes for 24 hours at the end of which time the mortality was recorded. The same general procedure and exposure time were followed throughout. A constant relative humidity chamber was employed in tests conducted at high relative humidities.

Approximately 100 specimens of each species killed by Dri-Die were examined under a wide field binocular microscope for external evidence of desiccation.

Table 1.—Comparison of the mortality of three species of adult female mosquitoes exposed for 1-hour to various dosages of Dri-Die.[a]

INSECTICIDE DEPOSIT (MG./SQ. FT.)	PER CENT MORTALITY (24 HRS.)[b]		
	A. quadrimaculatus	*C. fatigans*	*A. aegypti*
50	4	0	26
	0	7	20
	96	92	83
	96	87	78
100	0	13	48
	5	15	65
	100	90	92
	100	85	96
150	0	13	48
	18	77	42
	100	100	97
	100	100	96
200	100	57	54
	100	24	43
	100	100	100
	100	100	96
Controls	4	0	5
	0	1	6

[a] (Temperature range, 23° to 24.5° C.; relative humidity range, 67% to 76%.

[b] The specimens in the first two tubes at each dosage and in the first control tube were allowed access to a sugar solution immediately following the 1-hour exposure period and during the 24-hour holding period, whereas the remaining test and control groups were not.

RESULTS.—As shown in table 1, all species tested proved to be highly susceptible to Dri-Die dust. *A. quadrimaculatus*, the largest of the test specimens, was the most susceptible with exposures to as little as 50 mg./sq. ft. producing almost complete mortality. The mosquitoes having access to liquid immediately following exposure largely survived desiccation up to the level of 200 mg./sq. ft. at which point mortality was complete. Numerous specimens still living at the end of the 24-hour holding period were noticeably affected in that several of their legs were "flattened out" practically parallel to the resting surface. Such mosquitoes frequently died within 48 hours.

After having determined that a susceptible and a DDT-resistant strain (Okinawa) of *C. fatigans* were equally susceptible to Dri-Die, the former was utilized in the

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Table 2.—Mortality of *Anopheles quadrimaculatus* and *Culex fatigans* females exposed for 1 hour to various concentrations of Dri-Die.[a]

Species	Insecticide Deposit (mg./sq. ft.)	Total No. Specimens	Per Cent Mortality (24 hrs.)
C. fatigans	50	33	30
	50	22	9
A. quadrimaculatus	50	25	88
	50	24	33
C. fatigans	100	21	43
	100	54	68
A. quadrimaculatus	100	21	95
	100	24	75
C. fatigans	150	24	46
	150	24	70
A. quadrimaculatus	150	22	90
	150	22	86
C. fatigans	200	24	100
	200	25	84
A. quadrimaculatus	200	23	100
	200	20	90
C. fatigans	Control	27	18
A. quadrimaculatus	Control	23	13

[a] All specimens were kept at 100% relative humidity both during the exposure and 24-hour holding periods. (Temperature, 23° C.).

present tests (table 1). The susceptibility of this species was only slightly less than that of *A. quadrimaculatus* and it was better able to withstand the desiccating action of 200 mg./sq. ft. of the silica aerogel, with liquid available, than was *A. quadrimaculatus*.

Tests comparing the adults of the susceptible and DDT-resistant strains of *A. aegypti* revealed no significant difference in their susceptibility to the silica aerogel. The results given in table 1 show that the insecticide produces a mortality in this species of the same order as that in *C. fatigans*. However, *A. aegypti* was the least able of all species to take advantage of available sugar solution for rehydration. The 38 survivors of exposures to 150 and 200 mg./sq. ft. were re-exposed for 1 hour to 200 mg./sq. ft. of Dri-Die. At the end of 24 hours, all but two specimens were dead.

It was noted that a maximum relative humidity of 100% reduced the effectiveness of the insecticide to some extent although in certain instances, complete mortality in both *C. fatigans* and *A. quadrimaculatus* followed exposures to 200 mg./sq. ft. (table 2).

The residual effectiveness of silica aerogel dust was much greater than that of aqueous suspensions of the material at a dosage of 100 mg./sq. ft. (table 3). This differential in insecticidal activity disappeared at the level of 500 mg./sq. ft.

At high dosages, neither dust nor aqueous suspensions of Silikil-D showed appreciable effectiveness against *A. aegypti* until tested at a high relative humidity (95%), in which case the mortality increased substantially (table 4).

Some of the dead test specimens of all three species

Table 3.—Mortality of *Aedes aegypti* females exposed for 1-hour to Dri-Die dust and aqueous suspensions.[a]

Deposit	Total No. Specimens	Per Cent Mortality (24 hrs.)
Dust	27	100
	28	100
	25	100
	24	100
	25	92
	27	100
Suspension	24	37
	26	19
	24	41
	28	35
	23	26
	30	50
Control	32	11

[a] Immediately following preparation of the suspensions, they were applied to the test papers (100 mg./sq. ft.) and allowed to dry before use. Temperature, 24° C.; relative humidity, 72%.

exhibited in addition to complete desiccation, small hemispherical or spherical masses of body exudates which formed following 1-hour exposures to the silica aerogel (fig. 1). These were most frequently seen in *A. aegypti*, approximately one-third of the specimens containing them. The exudates were most commonly seen on the center of the scutum as dark red to tan masses and less frequently on the legs. These were found to be ninhydrin-positive. In *C. fatigans*, the exudates were generally transparent or semi-transparent, occurring on the wings, body or legs of certain specimens. Occasionally, oviposition was induced following contact with Dri-Die.

Discussion.—It has been shown by Ebeling & Wagner (1959) and others that silica aerogels are particularly effective in removing the protective lipid layer of the epicuticle of certain insects, following which there is a rapid water loss. If this same phenomenon applies to mosquitoes, the continuity of the lipid layer must be restored rapidly, at least in some species, since negligible mortality of *A. quadrimaculatus* occurred following 1-hour exposures to 50 to 150 mg./sq. ft., provided the mosquitoes were

Table 4.—Mortality of *A. aegypti* adult females exposed to Silikil-D at widely-separated levels of relative humidity (Temperature, 24° C.).

Insecticide Deposit (mg./sq. ft.)	Per Cent Mortality (24 hrs.)	
	42% R.H.	95% R.H.
250 (D)[a]	0	63
	0	13
250 (S)[b]	0	37
	11	29
500 (D)	21	42
	4	48
500 (S)	0	38
	3	61
Controls	4	11

[a] Regular dust form of the silica gel is designated as (D).
[b] Deposit applied by spraying papers with an aqueous suspension (S).

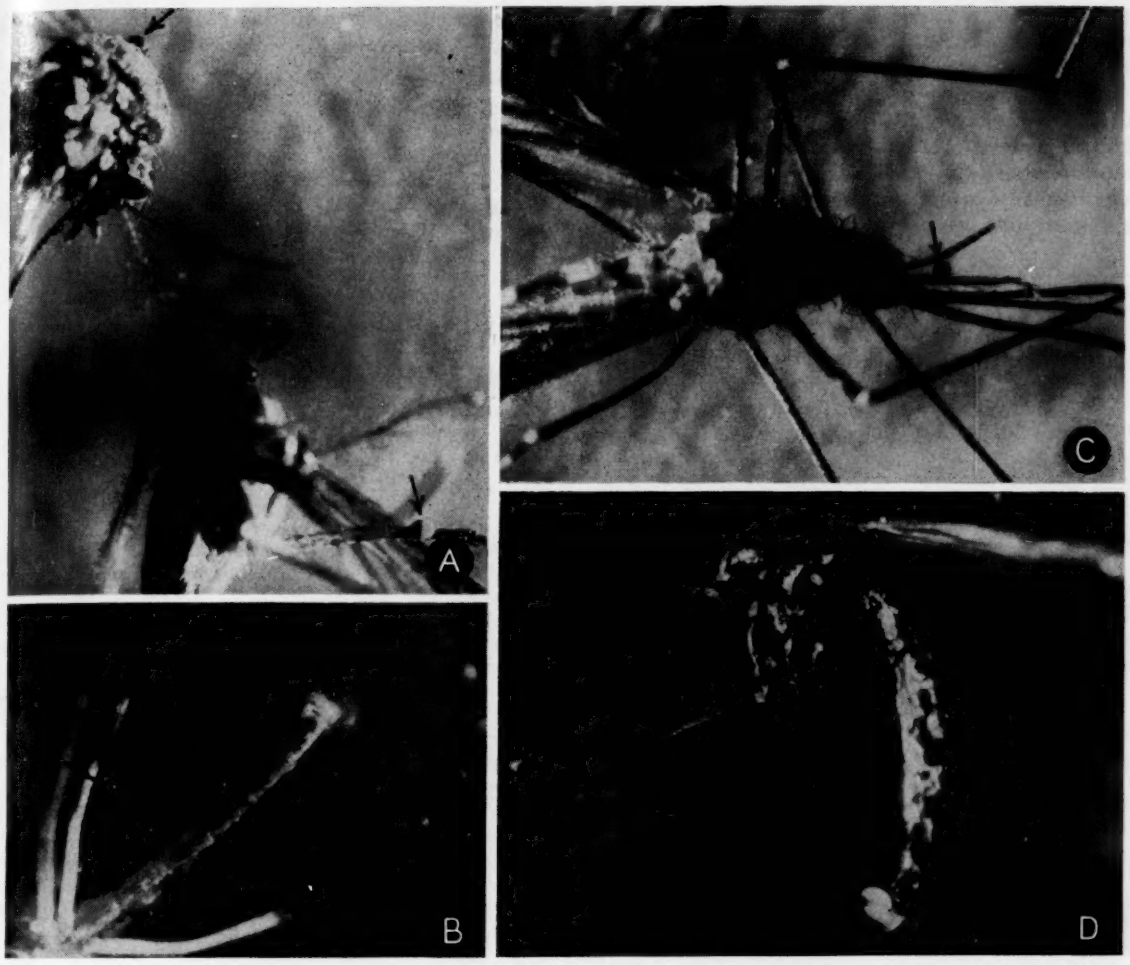

FIG. 1.—Effects of 1-hour exposures to the silica aerogel, Dri-Die: A, typical exudate masses on *A. aegypti*, those on the thorax being dark red and the one on the leg light tan; B, transparent spherical exudates on *C. fatigans;* C, exudate on antenna; D, induced or "forced" oviposition in *A. aegypti.*

given the opportunity to replace the lost fluid. However, following exposure to 200 mg./sq. ft. deposits, desiccation was irreversible. Of particular interest is the fact that in spite of the availability of a sugar solution immediately after the exposure period, approximately half of the *A. aegypti* died, the per cent mortality being unrelated to the amount of the insecticide deposit. This finding may have some connection with the more frequent formation of wax-like exudates in this species.

The effectiveness of Dri-Die dust against mosquitoes in a water-saturated atmosphere is especially striking and is in accord with the findings of Ebeling & Wagner (1959). They showed in studies with termites that fluoride-coated silica aerogel dusts produced almost the same mortality at 20% and 100% relative humidity. Unlike Dri-Die, which has a 4.7% ammonium fluosilicate coating over each particle, Silikil-D contains 1½% of this chemical impregnated throughout the entire particle. Therefore, the greatly increased insecticidal activity of Silikil-D against *A. aegypti* at a high relative humidity suggests that the water-soluble fluoride released may be the principal toxicant present.

The finding that silica aerogel residues deposited as aqueous suspensions were less effective than the dust is in agreement with the observations of Ebeling & Wagner (1959). It appears that suspending the chemical in water may cause a physical rearrangement of the particles so that they are less available than in the original form. However, in previous unpublished studies, we found that the application of a suspension at the rate of 250 mg./sq. ft. and above provided deposits which produced complete mortality in many tests. It appears, therefore, that suspending the silica aerogel in water does not reduce its insecticidal activity except in the way of interfering with the distribution of the individual particles. In this connection, it should be mentioned that attempts to distribute aqueous suspensions of Dri-Die evenly over filter paper were unsuccessful, largely because of the crude method used. It seems likely that higher kills could be obtained with more uniform deposits.

Following the transfer of the test specimens from the holding tubes to the exposure tubes, there was only a brief period of movement or flight activity following which the mosquitoes rested quietly on the treated surface. There

was no evidence whatever that any species tested was irritated by the deposits. Our earlier investigations with Dri-Die demonstrated repeatedly that the adult males of all species were more susceptible to the insecticide than females. Furthermore, blooded females were killed as readily as those used in the present studies. Considerable knockdown was noted at the higher insecticide dosages at the end of the 1-hour exposure period. Residues of Dri-Die dust on filter paper have retained their effectiveness over a period of several months of laboratory testing without any evidence of deterioration of the chemical.

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Relative Effectiveness of Resistance of Field Corn to the European Corn Borer, *Pyrausta nubilalis*, in Crop Protection and in Population Control[1]

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ABSTRACT

The survival and development of the European corn borer (*Pyrausta nubilalis* (Hbn.)) on 10 double cross hybrid varieties of field corn were studied in southern Minnesota in 1956, 1957 and 1958. Five of the varieties were resistant and five were susceptible to first-brood borer infestation. The following conclusions have been drawn: (1) The average survival and the average rate of development of the first-brood borers were lower on the resistant varieties than on the susceptible varieties. This fact indicates that plant resistance will reduce the injury caused by borer infestation. (2) While the average values showed the above difference, the reactions of each individual variety varied from year to year. Thus it was not possible to indicate the most resistant or the most susceptible variety in their respective groups. (3) Borer larvae suffered a mortality of nearly 60% during the first 2 hours, and nearly 80% during the first 24 hours of their life on the plant. This was true on both the susceptible and the resistant varieties. Thus, this initial mortality was perhaps not caused by host reactions. Based upon this fact, it was proposed that the borer mortality owing to plant resistance should be computed on the basis of the population that escaped the initial indiscriminate mortality. (4) The number of borers which were present during the following spring, *i.e.*, the beginning of the next annual cycle, was almost identical on both resistant and susceptible varieties. In other words, the differential found during the first brood (point 1 above) was later wiped out. This was brought about by an increase in the number of borers recorded in the fall as a result of higher second-brood infestation of plants that were resistant to first brood and had low first brood infestation and also by a lower overwintering mortality on the resistant than on the susceptible varieties. (5) The infection by a protozoan parasite, *Perezia pyraustae* Paillot, was lower among the borers on the resistant varieties than among those on the susceptible varieties. This relationship could have been responsible for the higher overwintering survival of borers on the resistant varieties.

The resistance of crops to their insect pests is a valuable tool in the protection of crop plants from injury by insects. First, it inflicts a high mortality among the insects which live on the resistant plants before or during the period of active feeding; this property will result in a protection of the current crop from severe insect damage. Secondly, the high mortality may produce a gradual reduction of the insect population in a general area throughout the period of a number of years, thus resulting in a population control of the insect pests.

The value of resistance of field corn to the European corn borer, *Pyrausta nubilalis* (Hbn.)[4], in reducing borer damage to corn has been well established. Recently, Everett *et al.* (1958) showed that in the absence of borer infestation, the borer-susceptible variety, Wf9×M14, produced higher yields than the borer-resistant variety, Oh43×Oh51A. Under moderate to high levels of initial borer infestation, however, the resistant variety yielded more than the susceptible variety. The latter fact was at least in part the result of a higher mortality of borers on the resistant variety during the season when plant injury was critical to the growth of corn.

It has been shown (Painter & Jones 1948) that an increase in the acreage of hessian-fly resistant wheat in an area resulted in a reduction of the population of hessian fly in the area. Thus it is not unreasonable to assume that since the borer-resistant varieties of corn inflicted a high mortality among the borers living on them, plant resistance would also exercise some degree of population control. This aspect, however, has not been critically evaluated. The real efficiency of plant resistance as a means of population control may be determined by the number of borers surviving at the beginning of the next annual cycle. The study of Everett *et al.* (1958), as well as many earlier works, reviewed by Painter (1951),

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[3] The authors wish to express their appreciation to Dr. E. L. Pinnel, Associate Professor of Plant Genetics, University of Minnesota (now head of the Department of Field Crops, University of Missouri) for the selection of the varieties used, and for calling our attention to the desirability of the present study from the standpoint of plant breeding. Sincere thanks are expressed also to Mr. R. E. Hodgson, superintendent of the Southern School and Experiment Station where the study was carried out. His generous assistance and excellent cooperation were greatly appreciated, particularly in view of the fact that keeping the experimental plots undisturbed throughout the winter inconvenienced the general farm management to some extent.

[4] Mutchmor & Beckel (1959) used the technical name, *Ostrinia nubilalis* (Hbn.) for the European corn borer. Until the taxonomic name for this insect has received further study we have used the name under which most of the literature appears.

Table 1.—Varieties, relative maturity and borer susceptible ratings of corn used in the study.

VARIETY	RELATIVE MATURITY (IN DAYS)	AVERAGE SUSCEPTIBILITY RATING[a]
Resistant varieties		
Minhybrid 411	112–116	1.9
Minhybrid 412	112–116	2.4
Minhybrid 414	110–114	1.9
Minhybrid 415	114–118	2.1
Minhybrid 511	107–111	2.5
Average		2.2
Susceptible varieties		
Minhybrid 408	113–117	3.6
Minhybrid 503	107–111	4.0
Minhybrid 507	105–109	3.6
Minhybrid 508	107–111	3.1
(WF9×M14)×(A257×A265)[b]	113–117	3.2

[a] The degree of injury on plants that resulted from a uniform infestation was rated on a 1 to 5 scale. The higher the number, the higher the susceptibility. The ratings are from a number of tests over several years by the Dept. of Agronomy & Plant Genetics, University of Minnesota.

[b] This is not a released hybrid variety. The maturity and susceptibility ratings are approximate values. This variety is indicated as WF9 cross in the later tables.

and more recently by Flemming *et al.* (1958), on the effect of plant resistance on borer damage and borer population, dealt only with the borer populations during the current season, and gave no attention to the populations surviving during the following spring.

The present study attempt to evaluate the efficiency of plant resistance as a means of population control of corn borer in addition to crop protection against this insect. This was done by artificially creating borer infestations of a given level on 10 different varieties of corn, and recording the borer survival during the current season and also in the following spring. The study was carried out first in 1956, repeated in 1957 and again in 1958, at the Southern Experiment Station, University of Minnesota, Waseca, Minnesota.

MATERIAL AND METHODS.—*Varieties of Corn.*—Ten double cross hybrid varieties of field corn were used in the study. The varieties, their relative maturity in number of days, and their borer susceptibility ratings are given in table 1. Five of the varieties are resistant and five are susceptible to corn borer infestation.

Plot Design.—Six replicates of each variety were planted. Each replicate was 6 rows wide and 150 feet long. The rows were 3½ feet apart, and the plants were 1 foot apart. The 10 varieties, each consisting of six 15-plant rows, were located at random within the replicate. All varieties were planted each year on the same day during the early part of the planting period normal for the area. The infested plants remaining in the experimental plot, after the summer and fall dissections, were not harvested or otherwise disturbed throughout the winter.

Artificial Infestation.—In order to ensure a uniform initial borer infestation on all the plants, three egg masses were placed on each plant during the period of first-brood natural oviposition in the field. The egg masses used were produced in the laboratory and averaged about 15 eggs per mass. The plants of different varieties showed only a slight difference in their average extended heights at the time of artificial infestation. The first-brood natural infestation was very low during the 3 years in which the study was made, and its effect on the experimental results was negligible.

Recording Larval Populations.—The basic procedure involved in determining the larval population on a plant was to dissect the entire plant and record the number and instar of the borers found. Throughout the year, the following three major checks were made:

1. Summer population. This check was made when the majority of the first brood larvae were in the mature stage. This was to determine the survival of the first-brood individuals. Six plants were dissected in each replicate and for each variety.

2. Fall population. This check was made after the corn had matured and usually after a killing frost. This was to determine the number of borers present at the end of the season which went into hibernation. These individuals included the residual first brood borers and the mature second brood borers. No attempt was made to differentiate between the two kinds of borers. Ten plants were dissected in each replicate and for each variety.

3. Spring population. This check was made after the spring thaw. Since the experimental plot was not disturbed throughout the winter, the mortality inflicted on the borers was the result of natural factors only. Ten plants were dissected in each replicate and for each variety.

In addition to the above records, borer populations were also checked each week during the first brood. Only one plant was dissected each week in each replicate and each variety.

It has been shown (Holdaway and Chiang, unpublished data) that the borer larvae suffer a very high mortality during the first few days of their life on the plant. In order to determine if this is true with the varieties under investigation, a supplementary study was made in 1957. Three plants of each variety were infested with three egg masses each. The exact number of eggs placed on each plant was recorded. The plants were dissected 2 hours, 18 hours, and 24 hours after the eggs hatched. At the time of dissection, the number of unhatched eggs on each plant was checked. Thus, the exact number of larvae hatched on each plant was known. The numbers of larvae present 2, 18, and 24 hours after hatching were then expressed in terms of percentage survival.

Checking Perezia Infection.—In order to investigate the possible relation between the frequency of infection of larvae by the Protozoan parasite, *Perezia pyraustae* Paillot, and the larval survival, the larvae which were present during the summer check in 1957 were collected and preserved in 70% ethyl alcohol for later diagnosis. Each larva was then crushed on a slide with clean forceps. The large pieces of body tissues were removed, and the smear was then covered with a cover slip, and examined under 430x magnification. The presence of characteristic, highly refractile spores was taken as evidence of infection.

RESULTS.—*1. Survival of the First-Brood Borers.*—The average number of borers per 100 plants of the different varieties found on various days during the first brood is presented in table 2. The average for the group of five

Table 2.—Survival of first-brood borers, indicated by the number of borers per 100 plants, on different varieties and on different days.[a]

	Susceptible Varieties						Resistant Varieties					
Date	408	503	507	508	WF9 Cross	Average	411	412	414	415	511	Average
						1956						
7/ 9	567	550	433	250	317	423	683	266	600	417	500	493
7/20	150	167	100	183	300	180	67	117	167	117	217	137
7/25	183	250	250	417	200	260	83	117	150	100	233	137
8/ 2	667	667	450	600	567	590	267	483	267	400	333	350
8/ 6[b]	362	337	346	396	265	341	192	252	275	200	310	246
						1957						
7/18	633	867	733	567	867	733	217	600	400	300	617	427
7/24	450	283	433	300	333	360	117	133	417	350	267	257
7/29	433	400	250	183	350	323	67	150	217	167	250	170
8/ 2[b]	189	139	133	147	147	151	67	83	178	142	133	121
						1958						
7/21	283	567	300	350	383	314	150	333	350	167	433	239
7/30	300	583	267	150	283	317	233	100	333	267	467	280
8/ 5[b]	219	225	166	141	233	197	69	136	156	136	164	132

[a] The plants in all varieties were infested with three egg masses per plant.
[b] Each number represents the average results of 36 plants. For other dates, each number represents the average results of 6 plants.

resistant varieties was lower than that for the group of five susceptible varieties on all dates and in all 3 years, excepting on July 9, 1956.

2. Development of the First-Brood Borers.—The average development of borers found on plants of different varieties and on various days during the first brood is indicated by instars in table 3. The average for the group of five resistant varieties was lower than that for the group of five susceptible varieties on all dates and in all 3 years, excepting August 2, 1956. The differences, even though small, show a rather definite trend.

The development of the borers can be measured by another method, namely the percentage of larvae that reached maturity on a certain day. The results are presented in table 4. Again the records show that the average per cent of mature larvae was lower on the resistant varieties than on the susceptible varieties.

3. Survival of Larvae During the First Day.—The number of larvae initially present on the plant (as indicated by the number of eggs hatched), and the number of larvae surviving at various times after hatching are given in table 5. The data show that regardless of the variety of corn, borer larvae suffered a mortality of nearly 60% during the first 2 hours, and nearly 80% during the first 24 hours of their life on the plant. These results confirm the findings of Thompson & Parker (1928) that borer larvae suffer a high mortality even under ideal conditions. The present data suggest that this initial mortality is perhaps not owing to any host reactions. The recognition of the nature of this mortality is important in evaluating the effectiveness of plant resistance, as will be elaborated later.

4. Survival of Overwintering Borers.—The numbers of borers per 100 plants found at the end of the first brood

Table 3.—Development of borers, as indicated by the average larval instars, on different varieties and on different days.

	Susceptible Varieties						Resistant Varieties					
Date	408	503	507	508	WF9 Cross	Average	411	412	414	415	511	Average
						1956						
7/ 9	1.0	1.0	1.1	1.1	1.0	1.0	1.0	1.0	1.0	1.0	1.1	1.0
7/20	2.2	1.9	2.3	1.5	1.5	1.9	1.7	1.6	1.1	1.9	1.8	1.6
7/26	2.6	2.4	2.7	2.0	2.7	2.5	1.8	2.6	2.0	1.5	2.6	2.1
8/ 2	3.6	3.4	3.1	3.3	3.2	3.3	3.9	3.2	3.4	3.5	3.4	3.5
8/ 6	4.0	4.3	3.9	4.0	4.1	4.1	3.8	3.7	3.7	3.8	4.0	3.8
						1957						
7/18	2.4	2.4	2.3	2.9	2.3	2.5	2.2	2.4	2.5	2.4	2.5	2.4
7/24	3.7	4.2	3.1	4.2	4.0	3.8	2.9	3.6	3.5	3.6	3.7	3.4
7/29	4.6	4.7	4.7	4.5	4.4	4.6	4.7	4.9	4.6	3.7	4.6	4.5
8/ 2	5.3	5.5	5.2	5.5	5.7	5.4	5.2	4.9	5.6	5.6	5.4	5.2
						1958						
7/21	3.0	3.0	2.7	3.1	2.6	2.9	2.3	2.8	3.2	3.3	2.7	2.9
7/30	4.4	4.3	4.1	4.4	4.3	4.3	4.1	3.9	4.8	4.1	4.1	4.2
8/ 5	4.6	5.1	5.0	4.9	5.0	4.9	4.8	4.9	4.8	4.6	4.9	4.8

Table 4.—Development of borers, as indicated by the per cent of borers which reached maturity, on different varieties.

	Susceptible Varieties						Resistant Varieties					
Date	408	503	507	508	WF9 Cross	Average	411	412	414	415	511	Average
8/6	13.2	20.4	10.2	14.7	15.7	14.8	9.8	8.3	7.6	10.4	12.8	9.8
8/2	55.9	66.0	56.3	64.1	75.5	63.6	41.7	43.4	65.6	33.3	52.1	47.1
8/5	34.6	31.2	33.3	27.5	27.4	30.8	24.0	34.7	25.0	18.4	27.1	25.8

Table 5.—Borer survival during the first day of hatching, 1957.

	Susceptible Varieties						Resistant Varieties					
Category	408	503	507	508	WF9 Cross	Average	411	412	414	415	511	Average
2 Hours After Hatching												
No. eggs hatched	48	37	51	39	55	230	36	42	47	43	43	211
No. larvae surviving	21	15	30	19	13	98	11	19	27	16	11	84
Survival (%)	43.8	40.5	58.8	48.7	23.6	42.6	30.6	45.2	57.4	37.2	25.6	39.6
18 Hours After Hatching												
No. eggs hatched	47	46	48	39	33	213	44	41	44	42	44	215
No. larvae surviving	10	17	10	8	16	61	11	24	3	17	24	79
Survival (%)	21.3	36.9	22.9	20.5	48.5	28.6	25.0	58.5	6.8	40.5	20.5	36.7
24 Hours After Hatching												
No. eggs hatched	41	34	45	38	42	200	43	38	41	49	40	211
No. larvae surviving	12	8	18	5	3	46	10	19	0	4	11	44
Survival (%)	29.3	23.5	40.4	13.2	7.1	23.0	23.4	50.0	0	8.2	27.5	20.9
Mortality (%)												
2 hours	56.2	59.5	41.2	51.3	76.4	57.3	69.4	54.8	42.6	62.8	74.4	60.4
18 hours	78.7	63.1	77.1	79.5	51.5	71.4	75.0	41.5	93.2	59.5	79.5	63.3
25 hours	70.7	76.5	59.6	86.8	92.9	77.0	76.6	50.0	100	91.8	72.5	79.1

Table 6.—Borer survival on the different varieties at three times during the annual cycle.

	Susceptible Varieties						Resistant Varieties					
Time of Count	408	503	507	508	WF9 Cross	Average	411	412	414	415	511	Average
1956–57												
Summer, 1956	362	337	346	396	265	341	192	252	275	200	310	246
Fall, 1956	112	79	55	124	133	100	52	64	65	67	52	60
Spring, 1957	27	20	23	21	15	21	17	10	22	27	29	21
1957–58												
Summer, 1957	189	139	133	147	147	151	67	83	178	142	133	121
Fall, 1957	97	110	69	96	108	96	83	73	167	102	156	116
Spring, 1958	12	19	6	12	6	11	12	17	21	8	6	13
1958–59												
Summer, 1958	219	225	166	141	233	197	69	136	156	136	164	132
Fall, 1958	25	40	23	55	32	35	20	33	33	27	32	29
Spring, 1959	13	3	2	2	3	5	3	5	10	3	3	5
Per cent mortality through the winter												
1956–57	75.9	74.1	57.3	83.2	88.9	78.8	67.6	83.8	65.3	59.5	45.5	64.7
1957–58	87.1	83.0	91.0	87.0	94.3	88.3	85.0	77.1	87.5	91.9	96.1	88.9
1958–59	46.8	91.7	93.1	97.1	89.6	86.9	83.5	85.0	70.0	87.6	89.6	82.8

(summer population), at the end of the growing season (fall population), and at the end of the overwintering period (spring population of the following year) on the different varieties are shown in table 6. As mentioned before, the fall population included both the residual first-brood and the mature second-brood borers. Since no attempt was made to distinguish the two types of larvae, the records of fall population do not indicate which of the borers present in the fall were survivors of the first-brood and which were second-brood borers. They do, however, represent the borer population that went into hibernation. They, compared with the population in the following spring, will show the survival of the overwintering populations.

Table 7.—*Perezia* infection among the first-brood borers, 1957.

Varieties	No. Borers Examined	Borers with *Perezia* Infection (%)
Susceptible		
408	44	72.7
503	20	85.0
507	31	64.5
508	35	48.6
WF9 cross	28	42.9
Average	158	62.0
Resistant		
411	4	50.0
412	22	68.2
414	24	54.2
415	42	45.2
511	26	53.8
Average	118	53.4

The results given in table 6 show the following two points: (1) While the average population at the end of the first brood was lower on the resistant varieties than on the susceptible varieties, the number of borers on both types of varieties in the following spring was surprisingly similar. (2) In 1957 and 1959, the practically identical spring populations resulted from a higher survival of the overwintering population on the resistant varieties than on the susceptible varieties. In 1958, the spring population was actually slightly higher in the resistant varieties than in the susceptible varieties. This resulted from a higher fall population in the resistant varieties, the high average fall population in the resistant varieties being caused by a higher fall population in three of the resistant varieties.

5. Perezia Infection among the Mature First Brood Borers.—The percentage of borers that showed *Perezia* infection is given in table 7[5]. There was a higher average incidence among the borers that matured on the susceptible varieties than among those on the resistant varieties. This correlation suggests that while plant resistance inflicted a higher mortality on the borers during their development, the borers which survived in spite of plant resistance were less vulnerable to protozoan parasitism. This finding may have some bearing on the higher survival of the overwintering populations on the resistant varieties than on the susceptible varieties, as will be discussed later.

Discussion and Conclusions.—*1. Plant Resistance as a Means of Crop Protection.*—It is generally known that under moderate to high borer infestations, resistant varieties of corn yield higher than susceptible varieties. Although no yield records were taken in the present study the value of plant resistance as a means of crop protection is clearly demonstrated by the fact that borers suffered a higher mortality on the resistant varieties than on the susceptible varieties (table 2). The present study, besides confirming the above relationship, reveals two additional points of importance.

The first point is the variability of results within the group. The survival of borers at the end of the first brood and the development of borers as indicated by the per cent of borers that reached maturity, are given in tables 2 and 4. To facilitate further analyses, pertinent records were pooled in table 8 and indicate the following: (1) The variety which showed the highest borer survival and the fastest borer development in the susceptible group varied in the 3 years. (2) The resistant variety which showed the slowest borer development also varied. The resistant variety which showed the lowest borer survival, however, was the same, Minhybrid 411, in all 3 years. (Minhybrid 411 is one of the two hybrids that showed the lowest susceptibility rating in the Agronomy tests over several years.) (3) A given variety may have the highest value in one year and the lowest value in another year. From these facts, it may be concluded that while survival and development of the borers were on the average, lower in the resistant group than in the susceptible group, it is difficult to indicate the most resistant or the most susceptible variety. This phenomenon is perhaps to be expected since the expression of plant resistance is influenced by environmental conditions which vary from year to year.

Table 8.—The varieties which showed the highest and the lowest borer survival and borers reaching maturity.

Year	Borer Survival at the End of First Brood		Borers Reaching Maturity (%)	
	Highest	Lowest	Highest	Lowest
	Susceptible varieties			
1956	508	WF9	503	507
1957	408	507	WF9 cross	408
1958	WF9 cross	508	408	WF9
	Resistant varieties			
1956	511	411	511	414
1957	414	411	414	411
1958	511	411	412	415

The second point is relative to the exact effect of plant resistance on borer survival. It was clearly shown that borers suffered a higher mortality on the resistant varieties than on the susceptible varieties. The results presented earlier will enable us to evaluate the effect of plant resistance more critically.

Pertinent records are assembled in table 9 for the following discussion. Each plant received three egg masses, with approximately 15 eggs each. Assuming that all of these hatched, there would have been 4500 larvae per 100 plants. The percentages of mortality computed on the basis of this initial population are given in No. 4 of table 9. It may be seen that plant resistance inflicted a mortality of only 0.6% to 2.2% above that on susceptible varieties (No. 5). This method of calculation has been commonly used to obtain the value of plant resistance. We believe that the value so obtained does not measure the true effect of resistance because it was shown (table 5) that the borer suffered a mortality of nearly 80% during the first 24 hours in the susceptible group as well as in the resistant. Thus the effect of plant resistance was exerted on the 20% of the larvae which escaped the initial mortality, instead of on the entire initial population. Hence the larval population, the survival of which was affected by plant resistance, would be about 900

[5] The assistance of Hubert Yamamoto, Research Assistant, Department of Entomology, University of Minnesota, in obtaining the results presented in table 7 is acknowledged.

Table 9.—Analyses of borer mortality on resistant and susceptible varieties.

No.	Category	1956		1957		1958	
		Susceptible	Resistant	Susceptible	Resistant	Susceptible	Resistant
1	Average initial number of larvae per 100 plants[a]	4,500	4,500	4,500	4,500	4,500	4,500
2	Average number of larvae expected at the end of 24 hours[b]	900	900	900	900	900	900
3	No. of larvae per 100 plants at the end of the first brood[c]	341	246	151	121	197	132
4	Mortality on basis of initial population (%)	92.4[d]	94.6	96.7	97.3	95.6	97.1
5	Difference in mortality owing to resistance		2.2		0.6		1.5
6	Mortality on the basis of population at the end of 24 hours (%)[e]	62.0	72.7	83.2	86.6	79.1	85.3
7	Difference in mortality owing to resistance		10.7		3.4		6.2

[a] On the basis of 3 egg masses per plant, and each mass containing 15 eggs.
[b] On the basis that about 80% of the initial population will perish regardless of variety (see table 5).
[c] See table 2.
[d] Example: $\frac{4500 - 341}{4500} \times 100 = 92.4$
[e] Example: $\frac{900 - 341}{900} \times 100 = 62.0$

(No. 2, table 9), instead of 4500 per 100 plants. On this basis the mortality owing to plant resistance would be 3.4% to 10.7% (No. 7, table 9), about five times as high as the first calculation. Furthermore, hatching was probably somewhat lower than the assumed 100%. Thus the larval population directly affected by plant resistance would be even lower than 900 per 100 plants, and the effectiveness of plant resistance would actually be a little higher than 3.4% to 10.7% as given. The effectiveness of plant resistance in reducing borer survival would be underestimated if the calculation were based upon the initial population. The evaluation made on the basis of the population that survived the initial indiscriminate mortality would be more accurate.

2. Plant Resistance as a Means of Population Control.—It was concluded from the results in table 6 that the resistant varieties under investigation were of no value as a means of population control. The question arises—"What are the mechanisms which by spring wiped out the difference in the size of populations which were present in the preceding summer?" Data in table 6 show that this lack of difference in the spring population was the result of two things: (a) a higher buildup of the population, prior to overwintering, in the resistant than in the susceptible varieties (in 1957), and (b) a lower mortality among the overwintering population in the resistant than in the susceptible varieties (in 1956 and 1958). The former could have been brought about in one or both of two ways: (a_1) A higher second brood infestation—Chiang, Holdaway, Brindley and Neiswander (in press) showed that plants that were more severely injured by first-brood borers received a lower second-brood infestation. Because the resistant varieties suffered less injury from the first-brood borers, they may have received a heavier second-brood infestation than the susceptible varieties. (a_2) A higher residual first-brood population—because the development of borers was slower on the resistant varieties, it is reasonable to expect that fewer borers pupated, and thus more first-brood borers remained to be present among the fall population on the resistant varieties than on the susceptible varieties.

The lower mortality among the overwintering population in the resistant than in the susceptible varieties (point b above) might have been related to the lower incidence of infection by *Perezia* among larvae in the resistant varieties (table 7). It has been shown that *Perezia*-infected borers have a lower survival than normal borers. Zimmack & Brindley (1957) reported a decrease of 21% in borer survival owing to *Perezia* infection, and Kramer (1959) reported a decrease of 59%. While both studies dealt with laboratory-reared nondiapausing borers it is logical to assume that the survival of the overwintering larvae in the field may be likewise affected by *Perezia* infection. Also Yamamoto (1956) found that in inbred lines of corn there was a tendency for a lower per cent infection by *Perezia* in borers reared on resistant lines than in those reared on susceptible lines.

It seems reasonable to conclude therefore that the tendency for borers that have fed on resistant corn to have a lower incidence of *Perezia*, and hence a greater ability to survive, is a factor contributing to equalizing the survival of borers on resistant and susceptible corn. The resistant varieties studied did not demonstrate any real value as a means of population control because the lower survival of the first-brood borers on the resistant varieties was later offset by a higher fall population and/or a lower mortality among the overwintering population on the resistant varieties than on the susceptible varieties.

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Loss in Corn Yield Associated with the Abundance of the Corn Leaf Aphid, *Rhopalosiphum maidis*, in Indiana[1]

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ABSTRACT

The corn leaf aphid is a common pest of corn in Indiana, causing occasional damage in a few widely scattered fields. In 1959, conditions favored the development of this insect and serious losses occurred in many corn fields in the northern two-thirds of the State. The area of heaviest infestation was a band three counties wide in the north central part of the State, from Warren and Fountain counties on the west to the Ohio line, and a single row of counties north and west along the Michigan line to Lake Michigan. The area of greatest losses followed a similar pattern. Losses in yield as high as 40% occurred in many fields.

The data obtained under these conditions of widespread and heavy infestations supported previous observations that severe infestations result in barren plants. In addition, such infestations also increased the production of nubbins. In one field, data indicated that light infestations can also reduce the yields as much as 10% through decreased size and weight of ears. There was also evidence that commercial hybrid corn varies in degree of infestation and tolerance to loss by this insect.

Data from the 12 areas of Indiana used for the Fall Corn Insect Survey indicated a high degree of correlation between loss of yield and per cent of plants infested. It was possible to estimate the amount of loss in individual fields from this relationship when the percentage of plants infested was known. The relationship of reduction in yield and infestation by the corn leaf aphid will probably vary from year to year as the interaction of control factors change, but it indicated the possibility of establishing a relationship of infestation and loss through the proper evaluation of environmental factors.

The corn leaf aphid, *Rhopalosiphum maidis* (Fitch), is present in Indiana cornfields every summer but usually becomes sufficiently abundant to attract attention of farmers in only a few scattered fields. In 1959 this insect was so prevalent in northern Indiana that growers became concerned about their corn crop.

Following receipt of numerous inquiries regarding this insect a survey of the situation in the northern part of the state was made in late July. This brief survey indicated that the aphid was more abundant than usual and that many fields had populations sufficiently high to result in injury to 5% to 10% of the corn plants. The heaviest infestations occurred in fields planted the latter part of May, while early- and late-planted fields had low or no infestations.

PREVIOUS REPORTS OF DAMAGE.—The importance of the insect and the effect it has on corn plants has been reported at various times since Fitch (1856) first described the species. However, until recent years estimates of loss have been restricted to a symptomatic expression of damage to individual plants. For a number of years following the describing of this species it was confused with the one attacking the roots of corn plants and the report of Forbes (1884) did not differentiate the two types of injury. Shortly after this, Forbes determined the two species as biologically different; in his 1905 report he listed the corn leaf aphid as causing leaves of corn plants to turn yellow or red and under heavy infestations leaves often shriveled and died. He also reported that the feeding of the insect on Broom corn resulted in a discoloration of the broom by a bacterial infection following the attack of the aphid. Sanderson in 1903 reported the corn leaf aphid injuring corn in Texas but did not give any details of the injury.

In 1921, McColloch reported in detail the injury observed on corn plants in Kansas. He differentiated these as (a) injury to the central tassel spike resulting in failure to shed pollen; (b) when aphids are exceptionally abundant, the lateral branches of the tassel were gummed up with honeydew which prevented pollen shedding; (c) tassels often failed to emerge completely; (d) molds and rots developed on the upper portion of the plant and often extended down to the ears; (e) in severe infestations corn leaves turned yellow and red, and often died; (f) aphids, feeding on kernels and silk, hastened maturity with ears only partially filled; and (g) the abundance of honeydew

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attracts corn earworm (*Heliothis zea* (Boddie)) adults often resulting in increased infestations from this insect. McColloch also observed differences in infestation on varieties of corn and indicated that Minnesota 13 was very resistant to the corn leaf aphid.

McColloch's (1921) observation on the increase in disease incidence following aphid attack is supported by the work of Brandes (1920). He successfully transmitted a mosaic disease of sorghum to sugar cane with this insect. The findings of Chardon & Veve (1923) showed that this insect was a vector of sugar cane mosaic, although the insect is not a primary pest of cane and does not develop high populations on this crop. The infestation occurs generally after weeding of the cane destroys the native grasses on which the aphid is feeding. Additional information on the possibility of this insect as a disease vector is reported by Oswald & Huston (1953). They found that barley yellow dwarf virus was transmitted only by aphids and included *R. maidis* in the group of vectors.

Wildermuth & Walters (1932) reported the insect as injuring barley and gave the first detailed study on the life history. Snelling *et al.* (1940) presented data on the resistance of varieties of corn and stated that barrenness produced by the feeding of the aphid was a physiological effect on the corn plant rather than a lack of pollination. They also noted that pollen production was reduced and that ears often failed to develop grain. Huber & Stringfield (1942) used aphid infestation to evaluate the resistance of inbred lines of corn to the European corn borer (*Pyrausta nubilalis* (Hbn.)). Neiswander (1948) cited the corn leaf aphid as of major importance as a pest of corn in Ohio and stated that infestations had been increasing over the past 15 to 20 years. He also reported that heavy infestations resulted in barren plants and indicated that weather might be an important factor in fluctuations of populations.

Bigger (1958) presented the first evidence of the effect of aphid infestations on corn yield by showing the per cent increase that occurs in barren plants during aphid infestations. He also gave data showing that commercial hybrids differed in the degree of infestation and in losses. Triplehorn (1958, 1959) mentioned damage to tassels, sooty mold growth, reduction in pollen shedding and barren stalks following aphid infestation. He also gave data on weight reduction per ear and per cent loss of yield in fields of corn of varying intensities of infestation. His data indicated that occasionally the degree of infestation was not closely correlated with loss of yield in individual fields.

EVALUATION OF DAMAGE.—The severe and extensive infestation of the corn leaf aphid in Indiana in 1959 offered the opportunity to observe and collect data on the effects of this insect on the corn plants and on the yield of corn. To obtain information on the effect of degree of infestation on the yield of dent corn, consecutive plants in a heavily infested field near Portland, Indiana, were tagged as showing no infestation, few aphids, and heavy populations on July 30 at the time the tassels were emerging from the whorl. Figure 1 shows a typical plant heavily infested with aphids. In October these plants were harvested and the ears separated by classes of infestation for study of yields. These data are given in table 1. The actual yield of 60 plants in each class of infestation is shown in figure 2.

Table 1.—Yields of corn from plants with varying degrees of infestation by the corn leaf aphid. Portland, Indiana, 1959.

		Class of Infestation[a]			Total Sample
		None	Light	Severe	
Plants in each class	No.	99	75	77	251
	%	39.4	29.9	30.7	100.0
Ears:					
Harvested	No.	102	76	43	221
Cobs barren	No.	0	0	16	16
Plants without ears	No.	0	0	18	18
Plants producing no seed	%	0	0	44.2	13.6
Yields:					
Weight of ears	Lb.	54.8	37.7	19.8	112.3
Weight of yield per plant	Lb.	.55	.50	.26	.44
Weight per ear	Lb.	.54	.50	.46	.51
Yield per acre	Bu.	72	65	34	57
Loss of total yield	%	0.	3.0	17.8	20.8
Reduction in yield within each class of infested plants	%	0.	10.0	53.2	—
Plants producing two ears	No.	3	1	0	4

[a] Classes of infestation defined as:
None —no aphids present
Light —small colonies or individual aphids scattered over plants
Severe—tassels dwarfed, heavy masses of aphids on tassels and upper leaves, presence of sooty mold growing in honeydew secretions on upper leaves, upper leaves showing a blighted condition, often extending down to the ear zone.
Classification of the infestations on the plants was made July 30. Harvest was made October 10 from plants tagged on July 30.

The severely infested plants in this field were easily identified by the blackened stunted tassels and in many cases, the dead upper leaves with the necrotic area extending downward toward the ear zone. Predators were exceptionally abundant. Coccinellids, both adults and larvae, lace-wings and syrphid flies predominated. In October, one ear of corn had 15 empty coccinellid pupal cases on the outer husks. In most of the heavily infested fields this abundance of predators was observable. For this reason insecticides at this period in the infestation should be used with the utmost caution to avoid destroying this predator population.

The infestation in this field averaged 60.6%, with about 30.7% of the plants heavily infested. The ears per plant harvested did not differ significantly in the non- and lightly-infested classes of plants. However, the severely infested plants had 23% of the plants barren (no ear produced) and an additional 21% with an ear that produced no grain (fig. 3). In this class of infested plants, 44% of the plants produced no yield. Based on the total plants harvested this represented a loss of 13.6% from nonproduction of seed.

An additional loss occurred in the reduction in the size and weight of the ears. In the plants with no infestation, the average ear weight was 0.54 pound. In the lightly and heavily infested classes the ears weighed 0.50 and 0.46 pound, respectively. On an acre basis the noninfested plants yielded 72 bushels of shelled corn. The lightly and heavily infested classes averaged 65 and 34 bushels per acre, respectively. The average yield of all classes was 57 bushels. If the yield of the noninfested plants is assumed to be the expected yield of this field in the absence of an aphid infestation, then the reduction in yield from the feeding of this insect was 20.8%.

There is the possibility that the use of noninfested plants growing in close proximity to infested plants may emphasize the loss that occurred, since those plants with normal growth might have an advantage over adjacent

FIG. 1.—Corn plant severely infested with corn leaf aphid. Note sooty mold beginning to develop in honeydew on tassel. Photograph by D. L. Matthew.

infested plants. However, there was no indication of plant stunting, other than the tassels, among the heavily infested plants. The within class loss of yield was 10% for the lightly infested plants and 53.2% for those classed as heavily infested. Of most significance from these data is the loss of yield in the lightly infested plants. Heretofore it has been assumed that if the corn leaf aphid infestation was not severe enough to produce barren plants, the loss in yield was negligible. These figures indicate that there was a measurable loss of yield from infestations by this insect at levels below those required to produce barrenness. Undoubtedly the environmental conditions were favorable to this insect in 1959 and conducive to survival and buildup of populations so that in less favorable years the loss from lightly infested plants may be lower. However, it does point out that this insect may cause severe losses with populations below those needed to produce barren plants and in years of severe infestations, the increase in barrenness is not the only loss in yield produced by this insect. It also emphasizes the fact that we may be underestimating the importance of many insect pests because we have not developed any method for measuring the losses they may cause at subepidemic populations.

LOSSES TO COMMERCIAL HYBRIDS.—A series of commercial hybrid dent corn varieties in a field near Bourbon, Indiana, was evaluated. Seven commercial hybrids were planted over a period of a few days in long strips ranging from 14 to 20 rows wide. Observations were made in this field in late September. An examination of 100 plants, (50 in each end of the field) in each variety showed all plants

FIG. 2.—Actual yield of 60 plants in each of the three classifications of infestation by the corn leaf aphid. Note the loss in the lightly infested class is represented by the same number of ears but smaller in size, while the loss in the severely infested class is owing to fewer ears and to those with only husks present. Photograph by G. E. Gould.

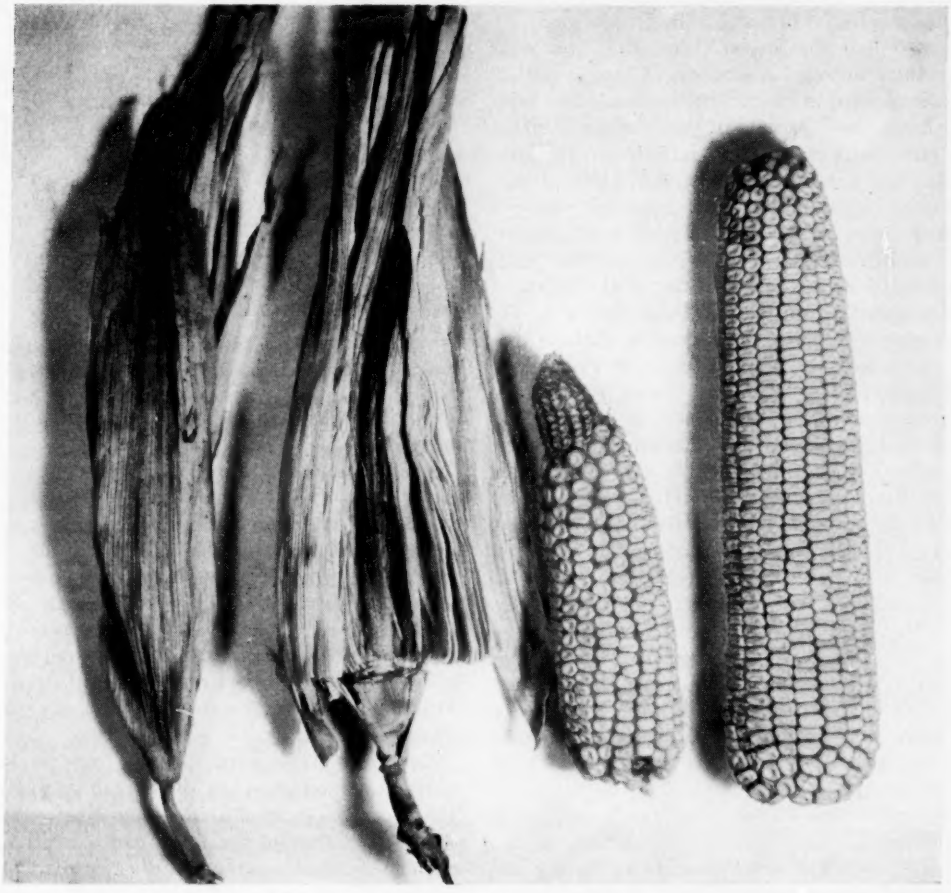

FIG. 3.—Types of yield loss from feeding of the corn leaf aphid compared with normal ear. The two barren ears show the normal development of husks and silk but the failure to produce seed either from lack of fertilization or a physiological effect on the corn plant. Photograph by G. E Gould.

infested with aphids. The severely infested plants were recorded, as well as those with nubbins or barren. Loss was estimated by assuming the nubbin-bearing plants represented a 50% loss and barren plants a complete loss. The total per cent loss was the sum of one-half the per cent of nubbin-bearing plants plus the per cent barren. The data from these hybrids are given in table 2.

The hybrid Crow 432 had a higher than average per

Table 2.—Infestation and losses to commercial dent corn hybrids from infestations of the corn leaf aphid. Bourbon, Indiana, 1959.

Variety	Per Cent of Plants			
	Severely Infested[a]	With no Ears	With Nubbins	Total Loss
Crow 432	47	7	18	16
DeKalb 423 (A)	32	14	12	20
(B)	38	18	12	24
Crib filler 166G	39	9	25	22
Indiana 610	26	21	14	28
Crib filler 151G	60	29	30	44
DeKalb 411	45	23	28	47
Crib filler 163G	68	34	33	50
Averages	45	19	22	30

[a] All varieties were 100% infested. The data in this column represents the per cent of plants showing severe infestation—stunted tassels, upper leaves dead and discolored with sooty mold, and massive areas of cast skins.

cent of plants severely infested yet showed the lowest loss. The hybrid Crib Filler 163G had not only the highest per cent of plants infested but also suffered the greatest loss. Indiana 610 had the lowest infestation and was slightly lower than average in amount of loss in yield. DeKalb 423 was planted in two different areas in the field and the data showed how similarly it performed as well as the degree of uniformity of the aphid infestation. DeKalb 411 had an average per cent of the plants infested but showed the second highest loss in yield.

It should be pointed out that the stage of development as well as the conditions of growth at the time the aphid infestation occurred could decidedly alter the relative degree of infestation as well as the losses in yield. To properly evaluate the inherent performance of these hybrids, they would have to be studied under controlled conditions. However, of significance from these data was the wide range of infestation as well as loss in yield which suggests that resistance to infestation and tolerance to losses in yield may be a promising line of investigation to reduce losses from the corn leaf aphid.

Regional Infestations in Indiana.—The Fall Corn Insect Survey in Indiana was carried out in late September and early October in 1959.[2] The long experience with corn insects of all the personnel assisting on this survey made possible the detailed observations recorded on the corn leaf aphid, the infestations of which are given in table 3. Figure 4 shows the counties comprising the regional areas used in the Indiana Corn Insect Survey. Numbers of sample corn fields were assigned on the basis of the acreage of corn in the counties with a minimum of one field per county and 10 fields per region.

In early September, observations and counts were made in a few fields with relatively high populations of the aphid. The damage to the corn plants was readily apparent and noninfested plants as well as those with low and severe infestations were easily identified. With these observations as a basis, it was decided to include the infestations and damage from the corn leaf aphid in the annual fall survey of corn insects.

The accompanying map, figure 5, shows the general distribution of the aphid infestation in Indiana. Those isolated counties in northern Indiana which showed no infestation, had some fields carrying very severe infestations, but these were not numerous enough to occur in the sampling.

Fig. 4.—The counties comprising 12 area units used in the Indiana Corn Insect Survey.

The heaviest infestation of the corn leaf aphid occurred in the northern three-fourths of Indiana (26.9%), while that of the southern fourth of the state averaged 6.2%. The proportion of heavily infested plants followed the same general pattern. The heaviest localized areas of infestation were the North Northcentral, North Northeastern, Northcentral and Northwest. The Northcentral area had the greatest percentage of plants heavily infested. The infestation was heaviest in the central third of the state with slightly less infestation in the eastern and western thirds, respectively.

Regional Losses in Corn Yield in Indiana.—In studying the effect of the 1959 aphid infestation on yield, The North Northwest area, North Northcentral, and the southern quarter of the State had a relatively high percentage of nubbins in the noninfested plants. In the northern areas there was a higher percentage of nubbin-bearing plants in the lightly infested class of plants accompanied by a considerable increase among the heavily infested plants. However, in the southern quarter of the state this condition did not exist, primarily because of the relatively low percentage of lightly infested plants and the almost entirely absent heavily infested ones. The proportion of

[2] M. C. Wilson, George E. Gould and David L. Matthew of the Department of Entomology assisted in collecting the survey data.

Table 3.—Corn leaf aphid infestation and damage. Fall 1959.

AREA	COUNTIES	FIELDS	PLANTS INFESTED[a] (%)			NUBBINS IN EACH PLANT CLASS[b]			BARREN PLANTS IN EACH PLANT CLASS[b]		
			Total	Light	Heavy	None	Light	Heavy	None	Light	Heavy
NNW	7	16	14.8	10.8	4.0	10.0	11.6	19.8	0.	1.0	3.1
NNC	5	9	25.8	15.6	10.2	7.1	7.2	23.1	0.	0.	12.0
NNE	6	10	32.0	28.4	3.6	0.	24.3	0.	0.	5.9	23.3
NW	6	15	29.1	23.5	5.6	4.2	11.5	50.6	0.	0.	9.4
NC	7	14	51.4	28.0	23.4	2.4	29.7	42.3	0.	0.	23.5
NE	10	19	30.7	19.4	11.3	3.0	25.9	44.0	.2	1.1	16.8
SW	8	13	20.3	12.9	7.4	.3	23.8	22.4	.4	0.	4.5
SC	7	11	24.0	15.3	8.7	3.1	22.8	32.1	0.	0.	14.7
SE	9	16	14.2	10.2	4.0	1.7	17.2	17.2	.3	2.1	0.
SSW	10	17	8.9	8.0	.9	9.3	4.4	1.5	0.	0.	0.
SSC	8	10	1.2	1.2	0.	10.1	0.	0.	0.	0.	0.
SSE	8	10	8.4	8.0	.4	14.2	0.	10.0	0.	0.	0.
			Summaries								
NN	18	35	24.2	18.3	6.1	5.7	14.4	14.3	0.	2.3	12.8
N	23	48	37.1	23.6	13.5	3.2	22.4	45.6	.1	.4	16.6
S	24	40	19.5	12.8	6.7	1.7	21.3	23.9	.2	.7	6.4
SS	26	37	6.2	5.7	.4	11.2	1.5	3.8	0.	0.	0.
W	31	61	18.3	13.8	4.6	6.0	12.8	23.6	.1	.8	4.2
C	27	44	25.6	15.0	10.6	5.7	14.9	24.4	.0	.1	12.6
E	33	55	21.3	16.5	4.8	4.7	16.8	17.8	.1	2.3	13.7
			State average								
			21.7	15.1	6.7	5.4	14.9	21.9	.1	.8	8.9

[a] Light —aphids present in small numbers.
Heavy—aphids present in sufficient numbers to cause stunted tassels, sooty mold on upper leaves from honeydew, and solid masses of cast skins.
[b] Per cent of plants in each class of infestation.

nubbin-bearing plants was highest in the Northcentral area closely followed by the Northeast, North Northeast, Southwest and Southcentral areas. In the class of heavily infested plants, the percentage of those plants producing nubbins was 50% in the Northwest and more than 40% in the Northcentral and Northeast areas.

Barrenness was practically nonexistent in the lightly infested class of plants. Only the North Northeast area showed any large amount of nonproduction in this class of infestation. In the heavily infested plants, barrenness averaged 23.3% and 23.5% in the North Northeast and Northcentral areas, respectively. The North Northcentral, Northeast and Southcentral averaged more than 10% barrenness. There was no barrenness recorded from the southern quarter of the state.

To obtain an estimate of the increase in nubbin-bearing plants and barren plants caused by aphid infestation, the percentage of these types of loss in the noninfested plants was subtracted from the per cent nubbins and per cent barren plants in the light and heavily infested plant classes. These net figures on nubbins and barrenness were then multiplied by the percentage of these classes of infestation. This gave the per cent increase in nubbins and barren plants for all plants in the sample. To convert this to a loss in production, one-half the increase in nubbins, (since a nubbin represented one-half a normal ear) and all the barrenness due to aphids were added to obtain the per

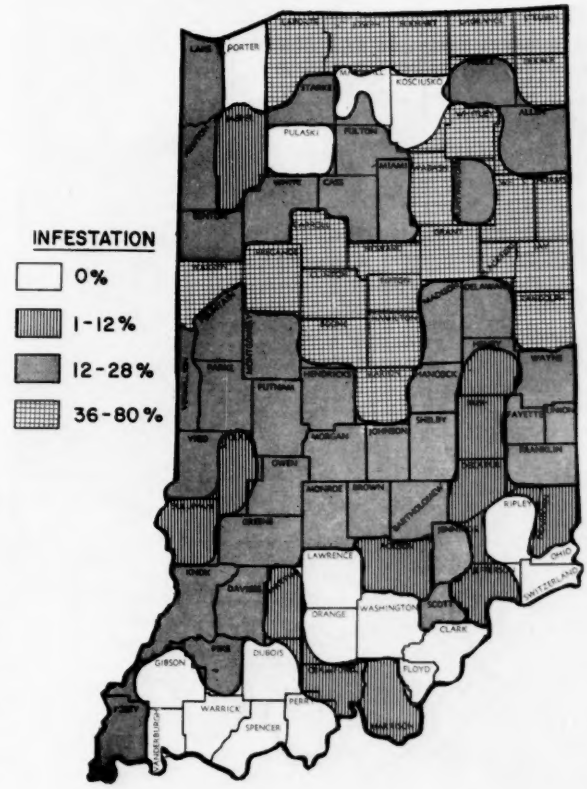

FIG. 5.—Generalized distribution and per cent of corn plants infested by the corn leaf aphid. Indiana Fall Survey—1959.

Table 4.—Corn leaf aphid infestation and losses in yield of dent corn. Indiana, Fall 1959.[a]

Area	Counties	Fields	Infestation (%)			Nubbins[b] (%)			Barren Plants[c] (%)			Per Cent Loss Due to:[d]		
			Light	Heavy	Total	Light	Heavy	Total	Light	Heavy	Total	Nubbins	Barren	Total
NNW	7	16	10.8	4.0	14.8	0.2	0.4	0.6	0.1	0.1	0.2	0.3	0.2	0.5
NNC	5	9	15.6	10.2	25.8	0.0	1.6	1.6	0.	1.2	1.2	0.8	1.2	2.0
NNE	6	10	28.4	3.6	32.0	6.9	0.	6.9	0.1	1.7	1.8	3.5	1.8	5.3
NW	6	15	23.5	5.6	29.1	1.7	2.6	4.3	0.	0.5	0.5	2.2	0.5	2.7
NC	7	14	28.0	23.5	51.4	7.6	9.3	16.9	0.	5.5	5.5	8.4	5.5	13.9
NE	10	19	19.4	11.3	30.7	4.4	4.6	9.0	0.2	1.9	2.1	4.5	2.1	6.7
SW	8	13	12.9	7.4	20.3	3.0	1.6	4.6	0.0	0.3	0.3	2.3	0.3	2.6
SC	7	11	15.3	9.8	24.0	2.5	2.5	5.0	0.0	1.3	1.3	2.5	1.3	3.8
SE	9	16	10.2	4.0	14.2	1.6	0.6	2.2	0.2	0.	0.2	1.1	0.2	1.3
SSW	10	17	8.0	0.9	8.9	0.	0.	0.	0.	0.	0.	0.	0.	0.
SSC	8	10	1.2	0.	1.2	0.	0.	0.	0.	0.	0.	0.	0.	0.
SSE	8	10	8.4	0.4	8.8	0.	0.	0.	0.	0.	0.	0.	0.	0.
Summaries														
NN	18	35	18.3	6.0	24.2	2.4	0.7	3.1	0.6	0.4	1.0	1.5	1.0	2.5
N	23	47	23.6	13.5	37.0	4.6	5.5	10.1	0.1	2.6	2.7	5.0	2.7	7.7
S	24	40	12.8	6.7	19.5	2.3	1.7	4.0	0.1	0.5	0.6	2.0	0.6	2.6
SS	26	37	5.7	0.5	6.2	0.	0.	0.	0.	0.	0.	0.	0.	0.
West	31	35	13.8	4.6	18.3	1.2	1.2	2.4	0.0	0.2	0.2	1.2	0.2	1.4
Central	27	44	15.0	10.6	25.6	2.5	3.4	5.9	0.0	2.0	2.0	3.0	2.0	5.0
East	33	55	16.5	4.8	21.3	3.2	1.3	4.5	0.5	0.5	1.0	2.3	1.0	3.3
State	91	134	15.1	6.7	21.7	2.3	1.9	4.2	0.2	0.9	1.1	2.1	1.1	3.2

[a] Losses estimated by subtracting the per cent nubbins and barren plants in the noninfested class from that in the light and heavy infested classes (table 3) and multiplying by the per cent light and heavy infestations, respectively.
[b] Ears one-half or less than the average size of ears on noninfested plants. Loss estimated as one-half this per cent.
[c] Plants with no ear present and/or those with husks present but no ear development. Loss estimated as total loss.
[d] Sum of one-half the per cent nubbins owing to aphid injury and all the increase in barrenness.

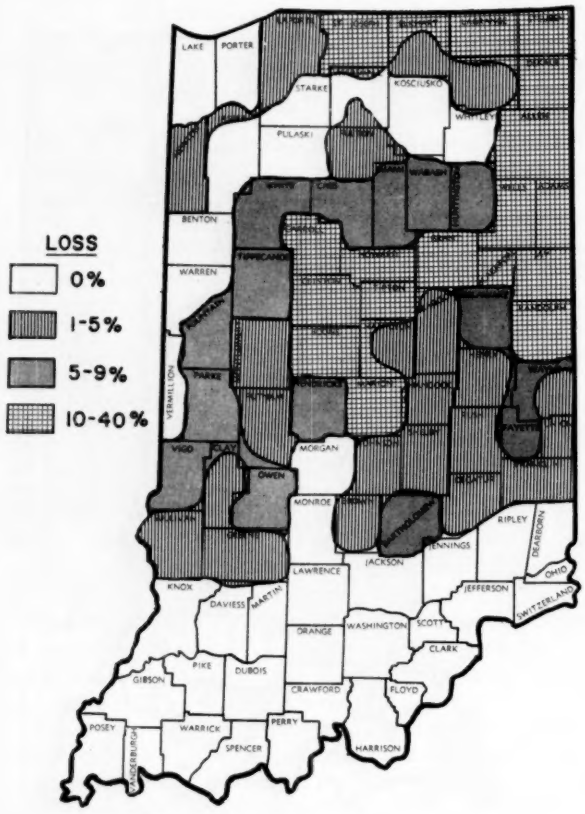

Fig. 6.—Estimated per cent reduction in corn yield from feeding of the corn leaf aphid. Indiana, Fall Survey—1959.

cent loss owing to aphid feeding. These estimated losses for the 12 areas in Indiana are shown in table 4.

The Northcentral area averaged the greatest loss, 13.9%. Economic losses occurred in the North Northeast and Northeast areas also. Losses of 2% or greater occurred in the North Northcentral, Northwest, Southwest, and Southcentral areas. Although the area of moderate infestation extended down the Wabash River Valley to the Ohio River, there was no measurable loss in the southern third of the state. The generalized losses for the state are shown in figure 6.

Relation of Loss to Infestation.—The data in table 4 indicate that the loss in corn yield closely follows the per cent of infestation for the 12 regional areas used in the survey. These data are shown graphically in figure 7 and indicate the closeness of this association. An analysis of the relationship gave a highly significant linear correlation coefficient of 0.920, and indicated that the degree of infestation accounted for 85%, (r^2) of the variability associated with loss in yield. When a second degree parabola is fitted to these data, the index of correlation is improved from 0.792 for the linear regression to 0.879 for the curvilinear. The error of estimates is reduced by the parabola 77.3%, compared with a reduction of 62.8% for the linear regression. Using the parabolic equation, $Y = 0.00066X + 0.00535X^2 - 0.20549$, the estimated loss from the field near Portland, Indiana, (table 1) amounts to 19.5%. This

conforms within the limits of error to the 20.8% loss in yield from observations made in this field.

The fact that this relationship is curvilinear points out several interesting facts. Below certain levels of infestation, few or no heavily infested plants are present. This may be because of unfavorable environmental conditions which inhibit colony development, or the migrating population of aphids was few in numbers and was distributed over a greater number of plants. In addition, the loss gradient becomes steeper as the percentage of infestation becomes greater. This is supported by the fact that as the general level of infestation increases, the proportion of heavily infested plants tends to increase and it is from these heavily infested plants that the greatest amount of loss occurs.

The data shown in table 4 also indicate that there is a high degree of association between the per cent of plants infested and the per cent showing heavy infestations. An analysis of these data showed a highly significant linear correlation coefficient of 0.881 and an index of correlation of 0.739.[3] A second degree parabola gave a slightly better fit with a correlation index of 0.788. The linear regression equation, $Y=0.42166X-2.51980$, gives an extrapolated estimate of 44.5% of the plants heavily infested when the per cent infestation reaches 100. The field containing the different corn hybrids listed in table 2, was 100% infested and showed an average of 45% of the plants heavily infested. However, when the 100% infestation level is reached, the percentage of heavily infested plants can continue to increase as conditions become more and more favorable to aphid development, so that a wider diversity in the per cent of heavily infested plants as the per cent infestation increases, may be expected. It is extremely doubtful that the plants in a cornfield would ever be 100 per cent severely infested.

Since the per cent of plants severely infested are highly correlated with the per cent infestation, it follows that there should be a high correlation with the per cent loss in yield. This relationship is also curvilinear and best expressed by the equation $Y=0.33505+0.27638X+0.01282X^2$. Comparisons of the constants associated with these three correlations are given in table 5.

The data in table 5 indicate that the loss is more closely associated with the per cent infestation than with the per cent of plants heavily infested. This may be explained by the fact that the lightly infested plants which are included in the overall per cent infestation contribute a portion of this loss. The larger B- and C- values for the parabolic function associated with loss and severely infested plants indicates that there is a much steeper gradient of loss associated with these plants than is associated with all infested plants. In the absence of heavily infested plants, there remains a loss of 0.33%, which may indicate the amount of loss associated with the lightly infested plants

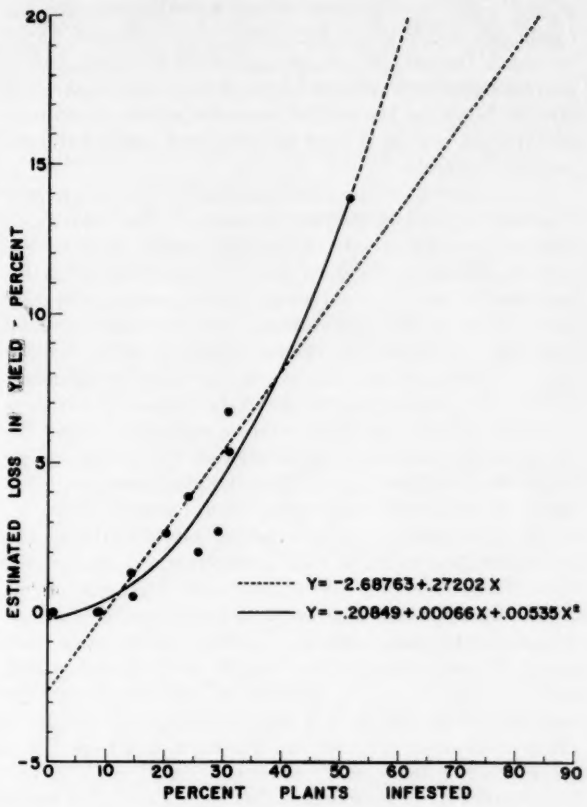

FIG. 7.—Losses in yield of corn and infestation by the corn leaf aphid. Indiana Fall Survey—1959.

[3] Index of correlation (rho) = $\sqrt{1-Sy^2/\sigma y^2}$.

Table 5.—Regression and correlation constants and measures of fit for the relationships between the per cent of plants infested, the per cent of plants heavily infested with corn leaf aphids and the per cent loss in corn yield. Indiana fall Corn Insect Survey, 1959.

REGRESSION	FUNCTION	A-VALUE	B-VALUE	C-VALUE	r	r^2	RHO	$e_{y.x}$	PER CENT GAIN OVER e_y[a]
Per cent loss in yield/% of plants infested	Linear	−2.68763	0.27202	—	0.92015	0.84665	0.792	1.48380	62.8
	Parabolic	−0.20849	0.00066	0.00535	—	—	0.879	0.90553	77.3
Per cent loss in yield/% of plants heavily infested	Linear	−0.49860	0.56049	—	0.90670	0.82210	0.772	1.60857	59.7
	Parabolic	0.33505	0.27638	0.02182	—	—	0.872	0.95742	76.0
Per cent of plants heavily infested/% of plants infested	Linear	−2.51980	0.42166	—	0.88135	0.77678	0.739	2.92588	54.7
	Parabolic	0.45727	0.09217	0.00649	—	—	0.788	2.44532	62.1

[a] $\frac{e_y - e_{y.x}}{e_y}$ = per cent improvement in the error of estimating Y by the regression.

at low levels of infestation and is immeasurable for the conditions under which these data were collected. In all instances, the parabolic function showed a closer relationship between the variables involved than did the straight line as shown by the reduction in the errors of estimate and the per cent gain over the standard error of the dependent variable.

CONCLUSIONS.—This relationship of loss to the per cent of plants infested by the corn leaf aphid is logically sound since without an infestation the insect could cause no loss in yield. However, there are many variables affecting this relationship, such as weather at critical periods, alternate hosts, time of first infestation, varietal resistance and tolerance, predators, and others, for which, at the present time, we have no quantitative measure of their influence. Whereas we can assume the general relationship of loss in yield and infestation, we should be cautious in applying the specific relationship as expressed by the regression equations from these data. These data indicate that infestation at levels which do not result in barrenness can decrease corn yield an appreciable amount. Variations in infestation and losses in yield in commercial corn hybrids indicate that breeding for resistance and tolerance may be a valuable approach to controlling losses from this insect. Although the data obtained in 1959 represent a wide range of observations under varied conditions of growth and weather, the exact amounts of loss associated with infestations of the corn leaf aphid may not be reliable for other areas or in other years. However, it is hoped that these observations and interpretations will result in further studies by other workers that may eventually result in a better understanding of the potential threat of this insect to the corn crop.

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Effect of Soil Treatments of Heptachlor and Parathion on Predators and Parasites of Root Maggots Attacking Rutabagas on Prince Edward Island[1]

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ABSTRACT

Applications of heptachlor placed along both sides of rows of rutabagas reduced pupal parasitism of the cabbage maggot (*Hylemya brassicae* (Bouché)) by *Aleochara bilineata* (Gyll.) more than 80%. At harvest, more than twice as many root maggot pupae and only one quarter as many parasites survived in the treated as in the untreated rows. In the insecticide-free region (a band about 4 to 5 inches wide around the plants) of the treated rows, there were approximately the same numbers of root maggots as in the untreated rows; the insecticide did not appear to affect the numbers of maggots attacking the plants. A similar treatment of parathion reduced the parasitism by 23% and caused no apparent reduction in root maggot populations. When applied in a 5-inch band in the row and just below the surface of the soil, heptachlor and parathion gave almost complete control of root maggots. The effect of such treatments on the predators and parasites has not been determined.

Although several species of insects such as *Aleochara bilineata* (Gyll.), *Trybliographa rapae* (Westw.), *Coenosia tigrina* (Fall.), *Scatophaga stercoraria* (L.), and various species of carabid beetles are known to destroy root maggot eggs, larvae, pupae, and adults (Perron *et al.* 1956, Read 1958a, 1958b, Wishart 1957, Wishart *et al.* 1957), little is known of the effect of these predators and/or parasites on root maggot infestations in the field, and, except for the observations of Morris (1960) that hepatchlor

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and aldrin had a detrimental effect upon parasitism, still less is known of the effect of insecticides on these beneficial insects. Where foliage sprays have been applied, root maggot injury has sometimes been more severe than where no insecticide was used (Read & Cannon 1958), and soil treatments with certain insecticides have resulted in an increase in the amount of root injury (Read 1960). Also, in Prince Edward Island, injury has often been much more severe in rutabaga fields where aldrin-fertilizer mixtures were applied broadcast than in neighbouring untreated fields. These observations indicate that insecticides may kill more predators and parasites than root maggots and allow root maggot populations to increase more rapidly in treated than in untreated areas. *A. bilineata* is considered one of the most important of the predators in that the adult beetles destroy root maggot eggs and larvae and the larvae destroy root maggot pupae inside the puparia. This paper reports on an experiment conducted to determine if certain applications of heptachlor and parathion would destroy *A. bilineata*, *T. rapae*, and carabid beetles in the soil and result in an increase in root maggot populations. When applied in a concentrated band below the surface of the soil in a ridged row, both insecticides gave highly significant control of root maggots attacking cruciferous crops (Read 1960). In this experiment the insecticides were applied along the sides of the rows in order that root maggots might survive and records of parasitism could be obtained.

METHODS.—Ten rows of rutabagas, each 400 feet long, were planted in ridges 4 to 6 inches high in a sandy soil at Kildare, Prince Edward Island, in an area adjoining a field that had been heavily infested with root maggots the previous season, and in which approximately 60% of the overwintering puparia were parasitized by *A. bilineata*. The seed was planted on June 5 and plants were allowed to develop without treatment with any pesticide until the first week of August. At this time, development of the first generation of the cabbage maggot, *Hylemya brassicae* (Bouché) had been completed (Read 1958b), and flies that would produce the second generation were beginning to emerge from the soil. On August 4, the area was divided into five equal sections, and six 20-foot lengths of row selected at random in each section were staked off. Duplicated treatments of heptachlor and parathion were applied to four of the plots in each section and the remaining two, which were at least 6 feet from a treated row, were retained as checks. Rates and applications of the insecticides are given in table 1.

The soil around the plants in the staked plots was examined periodically during the remainder of the season to determine the number of dead predators and parasites. The rutabagas were harvested during the last week of October without disturbing the soil around the plants. Blocks of soil, 10 feet long, 6 inches wide and 6 inches deep, were taken from the middle of the row in each plot, placed in wooden boxes, and taken to the laboratory for examination. Samples of the soil were similarly collected from the rows adjoining each treated row.

The root maggot puparia were removed from each box of soil using a flotation separator (Read 1958c). Each lot of puparia was then counted and the number parasitized by larvae of *A. bilineata* and *T. rapae* was determined by floating the puparia in a petri dish of water and examining them under a microscope; parasitized and healthy puparia are readily distinguished in that the parasitized ones almost always float with the dorsal side of the pupae facing upward and with the larval entrance holes and the parasite larvae on top of the pupae, whereas the healthy puparia float with the ventral side of the pupae facing upward. To determine the species of parasites in the puparia, adults were reared from samples of the puparia placed in moist soil under cages in the greenhouse.

Table 1.—Numbers of puparia of *H. brassicae* per 10 feet of row, and numbers and percentages parasitized by *A. bilineata*, after treatments with two insecticides applied in five sections of a field of rutabagas.

TREATMENT[a]	NUMBERS OF PUPARIA[b]		PER CENT PARASITIZED
	Total	Parasitized	
Section A			
Untreated	284	149	52
Heptachlor	390	52	13
Parathion	241	83	34
Section B			
Untreated	311	157	50
Heptachlor	211	35	14
Parathion	286	130	45
Section C			
Untreated	250	149	60
Heptachlor	220	30	12
Parathion	390	170	43
Section D			
Untreated	234	189	71
Heptachlor	243	44	15
Parathion	363	184	50
Section E			
Untreated	318	185	58
Heptachlor	303	31	10
Parathion	265	139	52

[a] Insecticides were applied at 5-lb. toxicant per acre in 6-inch bands on both sides of the row and worked into the top inch of soil. Heptachlor was obtained from the Velsicol Chemical Corp., Chicago, Ill.; and parathion, from Hopkins Agricultural Chemical Co., Randolph, Wisconsin.

[b] Average numbers from two 10-foot lengths of row in each section of the field.

RESULTS.—The results of the experiment, showing the effect of the treatments of hepatchlor and parathion on one generation of *H. brassicae*, are given in table 1. All plants in all of the plots were severely damaged by the root maggots, showing that neither of the insecticides provided any protection against root injury. However, heptachlor resulted in an 80% reduction in the number of parasitized puparia; there were more than twice as many healthy puparia and one-quarter as many predator-parasites surviving to produce a new generation of maggots in the heptachlor-treated plots as in the untreated plots. Parathion resulted in only a 23% reduction in the number of parasitized puparia. In the rows adjoining those treated with insecticide, the mean number of parasitized puparia was slightly lower than in the untreated plots. In column 2 of table 1 the figures also represent the number of larvae that completed their development in the roots and then pupated in the soil near the plants.

Periodic examinations of the soil during August and September showed that there were many more dead carabid and staphylinid (*A. bilineata*) beetles in and near the

rows treated with insecticide than in the untreated plots but, since it was difficult to find the dead insects in the soil, no attempt was made to count the numbers of predators in the plots.

Secondary species of root maggots, as the seed-corn maggot *H. cilicrura* (Rond.), comprised about 25% of the total number of puparia collected in the plots. These were parasitized to about the same degree as the *H. brassicae* in the treated and untreated plots. From rearings from the parasitized puparia of both pest species, about 99% were *A. bilineata*, the remainder being *T. rapae*. In 1958, six to eight per cent of the parasites were *T. rapae*.

DISCUSSION.—Although both heptachlor and parathion killed *A. bilineata*, *T. rapae* and carabid beetles under controlled conditions in the laboratory, such tests could not give a true indication of what occurs when the insecticides are used under field conditions. The results given in table 1 indicate that heptachlor, applied in the soil in bands along the sides of the rows, must have killed most of the predators and parasites, although accurate counts of the insects were not made. It would therefore be assumed that, since *A. bilineata* adults destroy root maggot eggs and larvae, there should have been many more root maggots in the treated than in the untreated plots. However, except for Section A in the field, approximately the same number of larvae completed their development in the treated as in the untreated plots. It was concluded that either the predators destroyed many root maggots before being killed by the insecticide, or that the insecticides killed some of the adults or larvae of the root maggots. In any event, there was about the same amount of damage in the treated as in the untreated plots, and from examinations of root injury only it would have been concluded simply that the insecticides were ineffective. However, analysis of the results given in table 1 shows that, since twice as many healthy puparia survived in the treated rows, the potential for production of a succeeding generation was more than twice what it would have been if no insecticide had been used, and there would be less than one-quarter as many *A. bilineata* to prey on the root maggot eggs and larvae and to parasitize the puparia. Theoretically, this would mean that, where insecticide was applied in the spring and there were three generations of root maggots during the season, there could be from 10 to 150 times as many root maggots in treated soil at the end of the season as in untreated soil.

On the same basis, assuming that broadcast applications kill the predators and parasites in the soil and also destroy many of the root maggots, it is probable that resistant strains of the insects soon develop (Finlayson *et al.* 1959, McClanahan *et al.* 1959). Since root maggots do not move about in the soil and are therefore subjected to a low concentration of insecticide, large numbers with a low degree of resistance may survive to produce a succeeding generation. However, the predators and parasites that move through the soil are subjected to a high concentration of insecticide and, although the survivors probably have a high level of resistance, a very small percentage may survive to produce a succeeding generation. Hence, four or five generations after resistance first becomes evident, there may theoretically be abnormally large numbers of root maggots and yet very few predators and parasites. This may explain why extremely severe damage by root maggots has occurred in areas in Prince Edward Island where broadcast applications of aldrin have been used for 2 or 3 consecutive years.

Although parathion applied in a concentrated band in the row effectively controlled root maggot infestations (Read 1960), the results obtained in this test indicated that this material did not have a marked effect on the predators and parasites. However, the material was applied to and worked into the surface soil and the results obtained may have been owing to nonresidual toxicity, since organophosphorous insecticides applied broadcast or in a 10-inch band and worked into the soil have generally been effective for not more than 3 to 4 weeks (literature reviewed by McClanahan *et al.* 1959).

Although considerable attention has recently been given to the problem of integrated control, studies have been mainly concerned with the use of foliage sprays (literature cited and reviewed by Smith & Hagen 1959). Control of soil pests presents a somewhat different problem in that it may be possible, by specific placement of the insecticide, to destroy the pest without drastically disturbing the biological control elements, particularly if the predators and parasites can survive by feeding on other nonphytophagous insects. Thus, by concentrating the insecticide against the pest, the grower is providing maximum protection to his crops, and is also providing conditions whereby the predators and parasites may be able to survive and destroy many of the pests not killed by the insecticide, and thereby conceivably prolong the propagation of economically important populations of insecticide-resistant strains of the pest. Much study is yet required on the relationship between beneficial and injurious insects and insecticides applied in the soil, but the results reported herein indicate that insecticides applied indiscriminately in the soil may destroy beneficial predators and parasites more effectively than pests, and may result in an increase in soil insect pest populations rather than controlling them.

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Effect of Sugarcane Borer Infestation on the Yield and Grade of Corn[1]

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ABSTRACT

The sugarcane borer, *Diatraea saccharalis* (F.), is a pest of sugar cane in portions of Louisiana. It was studied in an effort to determine the effect of infestation on the yield and grade of corn. Various degrees of borer damage were obtained by applying endrin granules on different schedules to corn growing in an area having a previous history of severe sugarcane borer infestation. High infestations of this insect caused a reduction in yield and grade of corn. Differences in yield resulted from (1) reduction in the number of primary ears which contained whole kernels, (2) a reduction in ear size and (3) a lowering of the weight per bushel of grain.

The sugarcane borer, *Diatraea saccharalis* (F.), is a pest of sugar cane, rice, grain sorghum and corn in Louisiana. Damage to sugar cane by this species has been evaluated. Arbuthnot (1958) showed an increase in corn yields when the plants were protected by insecticides. However, no study has been made of the effects of different levels of infestation on corn.

Damage by this species in continental United States is confined to south Louisiana, south Florida, and southeast Texas. Corn in southeast Louisiana (the Florida Parishes) is but little damaged by this borer and the reason is not known.

The species has been collected in corn as far north in Louisiana as Natchitoches Parish. The northermost area of severe infestation and the northern range of commercial sugar cane production in Louisiana is Rapides Parish. The areas of greatest damage coincide with the sugar cane and rice growing areas of Louisiana.

A study to determine the level of infestation required to cause damage to corn in Louisiana was initiated in 1957. Studies in 1957 and 1958 were of an exploratory nature designed to develop techniques and methods for making a comprehensive study of the effect of this species on corn. This paper is a report of the experimental work conducted in 1959 on this subject.

Tests in 1957 and 1958, using different insecticides, revealed that control of the sugarcane borer could be effected by the use of endrin. The granulated formulation was found to be superior to other formulations. No phytotoxic effects on the corn plant could be detected as a result of endrin application.

PROCEDURE.—The area selected for the 1959 study was the Rice Experiment Station at Crowley. Attempts in 1957 and 1958 to grown corn experimentally at this location proved unsuccessful due largely to an extreme infestation by the sugarcane borer.

Previous experience in working with corn had shown that unless the stand of plants was uniform, or very nearly so, the yield data could not be considered reliable. In order to obtain as uniform a stand as possible, recommendations by experiment station agronomists for land preparation and fertilization were followed. Seed of Louisiana 521, a white hybrid, treated with a dieldrin-thiram-methyl cellulose mixture for control of seedling pests was used. The field was planted as follows: A chain marked in 12-inch lengths was laid in the opened row and two seeds were carefully placed, one on either side, of each 12-inch mark at a depth of 4 inches. The seed was planted April 3. The plants were thinned to a single stalk 12-inches apart when the corn attained an average height of 6 inches, giving 80 plants in each row. A stand count at lay-by time showed practically a 100% stand.

The field was divided into a randomized block arrangement consisting of thirty-two 0.0193-acre plots. Each plot was 3 rows wide by 80 feet long and was bordered on each end by a 6-foot blank buffer.

The schedules of applications of 2% endrin in granulated form were as follows:

1. —1 application when the plants were 12 inches high.[4]
2. —1 application when the plants were 18 inches high.
3. —2 applications:
   No. 1. When the plants were 18 inches high.
   No. 2. At the immediate pretassel stage.
4. —3 applications:
   No. 1. When the plants were 18 inches high.
   No. 2. At the immediate pretassel stage.
   No. 3. At the ear-shooting stage.
5. —4 applications:
   No. 1, 2, and 3. Applied on the same schedule as listed for schedule 4 above.
   No. 4. In the dough stage.
6. —2 applications:
   No. 1. When the plants were 18 inches high.
   No. 2. At the ear-shooting stage.
7. —2 applications:
   No. 1. When the plants were 18 inches high.
   No. 2. In the dough stage.
8. Control—(No chemical treatment).

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[4] An average of 20% of the plants were infested with young larvae at the first application.

Table 1.—Plot yields—shelled grain.[a]

Schedule No.	Stalks Down or Broken (%)	Pounds Grain from			Per Cent of Grain Down	Pounds Grain from		Pounds per Measured Bushel	Grain Grade[b]
		Standing Plants	Down Plants	Total		Primary Ears	Secondary Ears		
1	22.5	18.6	8.1	26.7	30.4	23.2	3.5	56.95	3-W.C.
2	14.1	19.7	6.3	26.0	24.3	22.3	3.7	56.50	4-W.C.
3	5.6	27.7	3.3	31.0	10.5	27.0	4.0	57.58	2-W.C.
4	2.5	34.4	2.5	36.8	6.6	30.8	6.0	58.58	1-W.C.
5	3.6	30.4	1.9	32.2	5.8	28.0	4.2	58.30	1-W.C.
6	1.6	29.1	2.6	31.7	8.3	27.8	3.9	57.85	2-W.C.
7	5.6	26.7	2.1	28.8	7.3	24.6	4.1	57.45	2-W.C.
8	21.9	15.4	5.4	20.8	26.2	18.0	2.8	55.85	4-W.C.
L.S.D. @ 5%	7.18	8.90	2.79	8.64		5.34	3.76	0.52	
@ 1%	9.77	12.20	3.79	11.78		7.28		0.70	

[a] Weights corrected to a grain moisture base level of 14%.
[b] The grain grades were determined by Professor Russel Miller of the Department of Agronomy, Louisiana State University. W.C. = white corn.

Each treatment was replicated four times and all applications were made as scheduled. Application of the granules was made as follows: A 1-quart grain-sample can was attached to a 1-×2-inch stake 4 feet long, at approximately a 30°-angle. The lid of the can contained two uniform, precision drilled holes each approximately one-quarter inch in diameter. The can was filled with endrin granules and was held approximately 1 foot above the plants while the operator walked at a rate of four m.p.h. At this speed a dosage of 12 pounds of granules per acre was delivered. While the plants were small the stick was not used on the can. No additional jarring or shaking of the can was necessary. A uniform flow of granules occurred at all levels of can fullness.

Cans containing more but smaller holes, as well as other mechanical devices designed for experimental application of granulated insecticides were found entirely unsatisfactory insofar as accuracy of dosage and pattern of distribution were concerned.

Endrin granules in the 1957–58 studies had been found to control this species effectively in corn for approximately 10 to 14 days regardless of weather conditions. The schedules of applications used in 1959 were based on information obtained in 1957–58 and were planned to give various levels of borer damage at various stages of plant development.

RECORDS.—Records in several categories that were considered affected by borer infestation were taken:

Stalk Condition at Harvest.—When the grain moisture reached 20% the numbers of stalks per plot classed as "standing" and "down" were determined. A "down" stalk was one lying on the ground or one broken below the primary ear zone with the top portion either hanging or on the ground. Stalks commonly considered "lodged" (leaning but unbroken plants) were counted as normal standing stalks[5] (table 1).

Yield.—The yield from the center row of each plot was harvested when the grain moisture was 20%. All ears, regardless of size, which contained visibly developed kernels were harvested. Yields were separated according to primary and secondary ears and from standing and down stalks.[6] After harvesting, the ears in each category were placed in burlap bags and allowed to air-dry for 2 weeks. All yields were corrected to 14% moisture (tables 1 and 2).

Ear Damage by Borers.—The ears in each category were examined and the number visibly damaged by the sugarcane borer was determined (table 3).

Stalk Damage by Borers.—Ten consecutive stalks from the center of the middle row of each plot were cut at the ground line, stripped of leaves and topped at the node above the primary ear attachment. These stalks were split lengthwise and the total number of internodes (from the ground line to and including the internode above the node of attachment of the top ear) and the number of these

Table 2.—Total number of ears produced per plot and average size of ears.

Schedule Number	Number of Ears per Plot			Average No. Ears per Plant	Average Ear Size (ounces of grain)		
	Primary	Secondary	Total		Primary	Secondary	Mean
1	74	27	101	1.265	5.0	2.0	4.2
2	74	25	99	1.231	4.9	2.3	4.2
3	75	26	101	1.260	5.7	2.5	4.9
4	78	35	113	1.410	6.4	2.7	5.2
5	76	27	103	1.283	5.9	2.3	5.0
6	79	27	106	1.322	5.3	2.2	4.7
7	74	24	98	1.225	5.3	2.6	4.7
8	67	23	90	1.119	4.3	2.0	3.7
L.S.D. @ 5%	6.60				0.72	0.64	
@ 1%	8.99				0.97	0.64	

Table 3.—Borer damage to ears and stalks.

Schedule Number	Per Cent Damage to		Average No. Internodes per Plant[a]	Internodes Damaged (%)
	Primary Ear	Secondary Ear		
1	76.0	89.0	10.5	64.4
2	62.8	92.0	10.7	61.9
3	22.5	25.5	10.4	22.5
4	8.0	7.5	10.9	8.7
5	3.0	3.0	10.5	4.2
6	18.0	14.5	10.7	19.0
7	44.5	49.8	10.7	24.6
8	88.8	95.5	10.6	86.8
L.S.D. @ 5%	11.75	12.40		14.00
@ 1%	15.85	16.96		19.00

[a] Includes the number of internodes from the ground line to and including the internode of top ear attachment.

[5] One windstorm with gusts up to 60 m.p.h. occurred on August 20.
[6] When three ears occurred on a plant the upper two ears were included in the primary ear classification and the third ear classed as secondary.

internodes damaged by the borer were determined (table 3).

DISCUSSION OF RESULTS.—Results of these examinations are presented in tables 1 through 4. Data in table 1 show the effect of the various treatments in preventing stalk breakage. Treatment applied at the 12-inch stage did not affect stalk breakage later in the season. Application at this early growth stage may have prevented total loss of the plants, however, under conditions of a more severe infestation. Other than this early application, all treatment schedules resulted in significant reductions in stalk breakage. Data in table 3 show the relation between stalk breakage as recorded in table 1 and the percentage of joints damaged by the borer. Plots treated on all schedules except those at the 12-inch and 18-inch stages contained plants with relatively few damaged internodes. The extent of damage to individual internodes in these plots was also found to be much less severe than the damage occurring in the control and in the earlier-treated plots.

Primary and secondary ears were kept separated and both groups were examined for borer damage. All treatments reduced borer damage to the primary ears (table 3). Borer damage to the secondary ears was reduced in all treatments except those at the 12-inch and 18-inch stages. There was no difference in damage between the primary and secondary ears.

The number of primary ears produced per plot was influenced by borers (table 2). When insecticide applications were employed to reduce the infestation an increase in the number of primary ears resulted. The greatest number of primary ears was produced when the insecticide was applied during the early stages of ear development. The total number of secondary ears produced was not influenced by control of the borer population. The differences in the total numbers of ears produced were not significant.

The ear size (weight of grain) was also influenced by borer damage (table 2). The increase in weight of grain was greatest where infestations were maintained at the lowest levels. An increase in ear size may be expected since actual destruction of the kernels by the borers is involved as well as mutilation of the stalk and shank which may interfere with the transfer of plant nutrients and water. Effect of the borer population on size of the secondary ear was less than that on the primary ear. The increase in the number of primary ears and in the ear size accounts for a major portion of the increases in yield which resulted from several of the treatments.

The effect of the treatments on the yield of grain is shown in table 1. Control of the borer at any time from the immediate pretassel stage through the early ear development stage (schedules 3, 4, 5, and 6) resulted in increased yields. Application of insecticide to the plants at the time of dough stage of ear development (schedule 7) did not result in a significant increase in yield. These results indicate that control should be effected during the immediate pretassel and early ear development stages if maximum increases in yield are to be obtained.

Table 4.—Calculated yields per acre.

Schedule Number	Number Bushels from		Bushels Increase Over Check from	
	Standing and Down Stalks	Standing Stalks Only	Standing and Down Stalks	Standing Stalks Only
1	74.3	51.7	16.4	8.9
2	72.5	54.8	14.6	12.0
3	86.3	77.2	28.4	34.4
4	102.5	96.0	44.6	53.2
5	89.8	84.6	32.0	41.8
6	88.3	80.9	30.0	38.1
7	80.1	74.2	22.2	31.4
8	57.9	42.8		

A separation was made between the yields from the standing and down stalks since the yield from the standing plants only represents mechanically harvestable grain. Data in table 4 show the calculated total yields per acre and yields from standing stalks only. Where the level of borer infestation was high (schedules 1, 2, and 8) an average of 27% of the grain was down or nonharvestable mechanically. Plots which contained plants with low levels of borer infestation (schedules 3, 4, 5, 6, and 7) averaged 8% nonharvestable grain.

An increase occurred in the yield from the standing plants in all treatment schedules except those applied at the 12-inch and 18-inch stages (table 1). However, total yields (standing and down stalks) were significantly greater only where treatments were applied during the immediate pretassel stage and period of early ear development (schedules 3, 4, 5, and 6).

The grade of grain was influenced by borer infestation (table 1). Grain from the plants containing the lowest levels of borer infestations rated the highest in grade (schedules 4 and 5). As the infestation levels increased the grade was lowered.

According to the data derived from this study, infestations of corn by the sugarcane borer resulted in a reduction in the yield and in the grade of the grain. The reduction in yield which occurred resulted from (1) a reduction in the number of primary ears containing whole kernels, (2) a reduction in ear size and (3) a lowering of the weight per measured bushel of grain.

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Efficiency of Granulated Insecticides Influenced by Solvents Used for Impregnation[1,2]

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ABSTRACT

In mosquito larviciding and other aquatic insect pest control programs, quick kill of the larvae of the insects before they can change into pupae and adults is necessary. The use of granulated insecticides in such control measures has great possibilities, provided the formulations are capable of releasing a large portion of the toxicants within 24 hours after treatment. Formulations that release the toxicant slower are not desirable except possibly in certain prelarviciding treatments for mosquito control.

The efficiency of 44 petroleum solvents was investigated in a study of the various physical and chemical factors influencing and regulating the rate and magnitude of release of toxicants from granular formulations. The solvents had a wide range of physical and chemical properties. A 2% parathion-impregnated formulation was prepared using 30/60 mesh, AA-LVM attapulgite granules. The granules were dropped into water and the rate of parathion release was determined by bioassaying aliquots of the treated water against fourth-instar larvae of *Culex quinquefasciatus* Say.

The rate and magnitude of release for almost all of the petroleum solvents (aromatics, paraffins, and naphthenes) were found to be affected to a great extent by the flash point, minimum and maximum boiling points, distillation range and evaporation rate of these solvents. It was further noted that there existed an inverse relationship between the rate and extent of release and these physical characteristics of the solvents. With an increase in the values of these properties there was a tendency for slower releases and vice versa. A simple formula has been presented which illustrates this relationship, as follows:

$$REI = FP \times IBP\ (EP\text{-}IBP)\ 10^{-5}$$

Where REI is relative efficiency index, FP is flash point, IBP is initial boiling point, and EP is end point. Solvents with higher REI values generally resulted in formulations releasing parathion slower than those prepared with solvents having lower REI values. Solvents with extreme distillation properties are easily separated by this formula.

By selecting suitable solvents to impregnate granules with the toxicant solution, it is possible to prepare a wide array of granular formulations which will give either slow, intermediate, or rapid release of the toxicant depending on the particular solvent. The studies reported are aimed at gross separation of petroleum solvents into highly efficient, less efficient and inefficient solvents which might be used for impregnation of granular carriers with parathion.

Granulated formulations of insecticides are complex in nature. Their use for specific pest control purposes requires a knowledge of the basic physico-chemical characteristics of each of the ingredients in such formulations. Mulla & Axelrod (1959) pointed out factors that influence the performance of various types of granulated insecticides in mosquito and other aquatic insect pest control programs.

There are two major types of granulated insecticides marketed for both experimental and routine control treatments. One is the type in which absorbent inert particles are impregnated, with or without an organic solvent as a carrier, with the toxicant. The other type consists of inert particles, not necessarily absorbent, that have been coated with a dust, powder, or wettable powder form of the toxicant. The impregnated type of granular formulation constitutes a great portion of the granulated insecticides produced commercially. During the past decade this type of formulation exclusively has been employed in the investigational phases as well as in large scale control operations directed against mosquitoes, aquatic gnats, midges, and many agricultural pests.

Granulated insecticides have certain advantages over sprays in mosquito and other aquatic insect pest control programs in which the formulation should penetrate the emergent vegetation and plant canopy over breeding grounds. The residue on food and forage crops is smaller when granulated insecticides are used than when spray insecticides are used. This feature is one of the desirable attributes of granulated insecticides for controlling mosquitoes and aquatic gnats and midges breeding in water covered with aquatic weeds or food and forage stands. In trials comparing granulated BHC (30/60 mesh) applications with sprays on the first and second cuttings of alfalfa, lower residue levels for the granulated materials were indicated on both cuttings (Ware 1959).

Whitehead (1951) pointed out the utility of granular materials for the control of *Psorophora* mosquitoes in rice fields. It was believed that granulated insecticides would penetrate the plant canopy better than the sprays and would drop into the water where mosquitoes were breeding. However, field trials with parathion and dieldrin granules (15 mesh attapulgite) prepared with xylene as the impregnating solvent showed that granular formulations gave poorer control of mosquito larvae than sprays. This failure was attributed to slow release of the toxicants from the granules. In subsequent tests, good results were obtained with other combinations of carrier and toxicants. Better and longer control of *Culicoides furens* (Poey) was obtained in a densely foliated marsh with granulated BHC and dieldrin than with their sprays (LaBrecque & Goulding 1954). Keller *et al.* (1954) initiated field trials with various granulated toxicants against salt marsh mosquitoes. Some of these formulations were shown to be equally as good as sprays at the higher dosages while others proved inferior to sprays.

LaBrecque *et al.* (1956) carried out laboratory studies

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[2] The names of firms manufacturing equipment or producing the solvents used in the experiments are included for descriptive purposes. No endorsement of such products is implied. All the information pertaining to the physico-chemical properties of the solvents was furnished by the suppliers of these solvents, to whom the authors are grateful.

by impregnating attapulgite granules with dieldrin at 1% concentration using highly volatile and less volatile solvents. No difference in the magnitude of release was attributed to the various solvents. It is possible that the high dosages of the toxicant used in the tests might have overshadowed any real differences that might have existed among these formulations. Addition of the test insect (mosquito larvae) to the water containing granules and their contact with the toxicant pellets in these tests would yield good kill but not provide a good measurement of the rate or extent of release of the toxicant into the water column. Weidhaas (1957) conducted laboratory studies on the release of parathion from granular carriers using acetone, benzene, and Velsicol AR-60 as solvents for impregnation. The magnitude of release in no case was over 48% after 1 day for the 1% parathion formulations with any one of the carriers. The magnitude of release was quite low as indicated for the 10% parathion formulation. It was pointed out that solvent AR-60 reduced the release of parathion from its granules.

Chapman (1955) obtained good results with some granulated insecticides and poor results with others against *Mansonia* larvae. These mosquito larvae obtain air from the roots of submerged plants and therefore do not ascend to the surface of water for air intake. Controlling the larvae with spray application was rather difficult and tests with granulated EPN proved that this formulation yielded excellent control. Dieldrin, parathion, lindane, endrin, and DDVP granules reportedly yielded poor control of the larvae. Rogers & Rathburn (1958) developed a paris green granular formulation on vermiculite for surface feeding species of *Aedes*, *Anopheles* and *Psorophora*. This formulation, however, is considered more a coated than an impregnated granule and because of its low bulk density would remain on the surface of the treated water. Webbe (1955) obtained excellent control of anopheline and culicine larvae by using 1% dieldrin granular formulation (at the rate of 10 ounces of actual toxicant per acre) in swampy breeding grounds. In these trials no studies were made on the comparative effectiveness of sprays versus granular formulations of dieldrin. The swamps were 20% to 80% covered with dense and tall vegetation, and it was justifiably assumed that spray applications would penetrate the plant canopy poorly.

From the review of the literature it is apparent that granulated insecticides have been studied mainly for their efficiency and practicability in mosquito, gnat and midge, and agricultural pest control. Although a wide variety of granular formulations has been made available for both general use and investigational purposes, in most cases the ingredients of the formulations have not been reported. Critical evaluation of granular formulations versus sprays has, therefore, not been accomplished. In a few studies comparing the efficiency of granular versus sprays, higher dosages of the toxicant have usually been employed. No dosage response lines have been determined for either one of these formulations in field or laboratory studies.

The role of each of the various ingredients that go into a granular formulation has not been adequately studied. Each one of these ingredients has unique physico-chemical properties that would affect the efficiency of granulated insecticides in a variety of pest control programs. This paper deals with a detailed study of the role played in the performance and efficiency of granulated insecticides by some organic solvents used for impregnation.

MATERIALS AND METHODS.—The granular formulation prepared for all the tests contained 2% parathion impregnated on 30/60 AA-LVM attapulgite granules (Floridin Company, Tallahassee, Florida). The 30/60 mesh granules were selected because more efficient formulations were thought to be developed with this size carrier than with the coarser meshes. The disadvantages of this size, however, are the presence of some fines and the small size of the particles. This carrier as a base would not differentiate between solvents having close physical properties. For evaluating solvents with close physical properties, coarser mesh granules are probably more desirable.

The solvents for impregnation were used at a concentration of 10% based on the weight of the finished formulated product. A batch of 40 grams of the carrier was used per sample. Evaluations of the rate of release of parathion from the fresh formulations were made within 3 days after preparation. After the first test, the samples were stored in paper bags at room temperature, ranging from 60° to 80°F. Representative samples were retested after storage periods varying from 10 to 21 weeks for ascertaining the pattern of release after storage. It was assumed that most formulations made available commercially would be used for pest control within this period.

Preparation of Granules and Formulating Equipment.—A small, rotary tumbling unit (fig. 1) was designed for preparing the small samples of granular formulations used in the tests. The motor unit (Bodine Electric Company

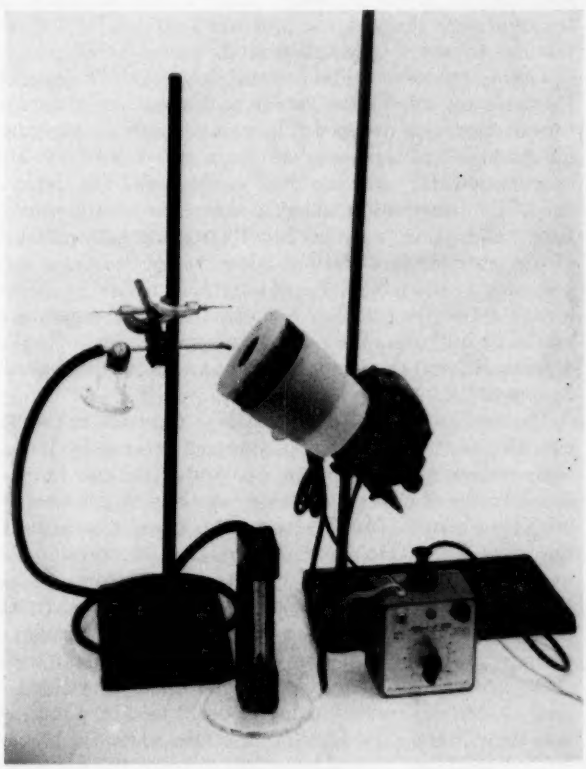

FIG. 1.—Equipment for preparing granular formulations, showing speed controller, motor, tumbler, atomizer, and air flowmeter.

Type NSE-11R) was provided with a right-angled drive maintaining 4 in.-lb. torque under normal operating conditions. The motor speed at the output shaft was reduced by a ratio of 72 to 1. A 1-pint plastic refrigerator cup with a metal disc was coupled with the drive of the motor. For impregnating granules, other cups of the same size were easily slipped into the fixed cup. The free cup (dark cup in fig. 1) was pushed tightly into the fixed cup so that slippage during tumbling and rotating was avoided. A lid with a central hole for directing the spray onto the granules was found desirable.

The speed of the tumbling unit was regulated by a variable transformer (Standard Electrical Products Company, Dayton, Adjust-a-Volt Type PA-1). The speed of the rotary unit was maintained at 50 r.p.m. while impregnating and mixing the granules. The rotary unit was set at an angle of 40 degrees on the vertical plane for a 40-gram batch of granules. (This angle varies according to the amount of granules to be impregnated: the angle increases for larger amounts and decreases for smaller.)

The toxicant solutions were sprayed onto the granules through a small atomizer (DeVilbiss No. 127). By means of a flowmeter (Matheson Company, Model No. 215A), the air volume delivered through the atomizer was kept constant at 3,500 ml. of air per minute.

The toxicant solution was prepared by dissolving 0.9 gram of technical parathion in 5 ml. of each solvent to be tested. The 40 grams of granules were impregnated with the solution while the rotary unit was in operation and an additional 1 ml. of the solvent was sprayed onto the granules through the atomizer to remove traces of the toxicant left in the atomizer. After all the solvent and toxicant were sprayed, the unit was tumbled for 3 more minutes to insure thorough mixing.

Testing Procedure.—To ascertain the rate of release of the toxicant, small quantities of each parathion granular formulation were dropped into a mayonnaise jar containing 3,500 ml. of tap water having a pH of 8 to 8.5 and creating a water column of 8.25 inches high. The quantities of the formulation added to the water usually varied from 0.85 to 1.05 grams per jar. By adjusting the amount of the granular formulations added to the water, it was possible to obtain 5 p.p.m. of parathion if it were all released from the carrier particles. Since temperature markedly influences the rate and extent of release (Mulla & Axelrod 1960) the jars were kept at a constant temperature of 85° F.

The rate and magnitude of parathion release at the 8-, 24-, 48-, and 72-hour intervals were measured by bioassaying aliquots from the treated water against fourth-instar larvae of *Culex quinquefasciatus* Say. Aliquots of 10 ml. were aspirated from the top and bottom (1 inch above the granules) of the water column and composited for final dilutions. Depending on the type of formulation (with good release or poor release), a volume of 0.25 to 0.4 ml. of the aliquot was added to 100 ml. of tap water in a pint-sized ice cream cup to which 25 fourth-instar mosquito larvae had been added. These dilutions yielded a final theoretical concentration of 0.012 to 0.02 p.p.m. of parathion in the cups. In a few instances where the granular formulations had a lower capacity of releasing the toxicant, higher final theoretical concentrations (0.05 to 0.08 p.p.m.) were maintained. Each cup was replicated three times and the cups containing the larvae were kept at 75° to 78° F. The larval mortality was read 24 hours later.

The per cent release for each formulation was calculated from the observed mortality of the larvae in the cups. The amount of toxicant present in each cup was determined from standard dosage mortality lines established for parathion against the test insect. In this way the per cent release of parathion in the initial solution was ascertained.

Tests have indicated that magnitude and rate of calculated release vary with the concentration of the toxicant in the formulation and concentration of parathion added to the water in the jar and in the cups. Thus, adding parathion granules to the jars at a concentration of 0.1 to 1 p.p.m. of parathion would yield greater and faster release of this toxicant than when it is used, as here, at 5 p.p.m. concentration. It has also been observed that obtaining kills above the 90% level usually indicated higher releases as compared with release calculated at the lower end of the dosage mortality line. Combinations of carrier and solvents without the toxicant were also tested in a few cases, but no mortality was caused by them.

RESULTS.—The petroleum solvents used in the experiments were obtained from various commercial sources. Since a large number of petroleum solvents are available to the pesticide-formulating industry, testing and evaluating all or a great part of the total in granular formulations would require a long time. For quick evaluation, samples of representative solvents were obtained from several petroleum firms. Physical properties of some of the solvents as reported by the various suppliers have been given so that a possible correlation between speed of release and these properties could be attempted.

Chemical composition of only a few of the solvents has been roughly determined by means of mass spectrographs. The fractions are known only insofar as the types or classes of compounds are concerned. Composition data for a few of the solvents are given. The chemical composition, of course, will determine the physical properties of these solvents. The solvents studied by no means exhaust the list of available solvents. There undoubtedly are other solvents whose efficiency would be equal to or greater than the efficiency of those tested.

AR Series of Solvents.—Four aromatic types of Velsicol solvents were evaluated; some of the physical properties of these solvents are included in table 1.

The magnitude of release at each time interval varied from solvent to solvent, but was lower for the three low-volatile solvents than for the highly volatile solvent AR-35 (fig. 2). The trend and extent of release followed the same pattern for AR-60, AR-55 and AR-50, both in the fresh and aged formulations. However, a decreased rate of release was detected for the aged AR-35 formulation in comparison with the fresh formulation.

These findings are in agreement with the theory that a solvent of low volatility carries the toxicant to the inner portion of the carrier particles. The solvent remains in the granules for many weeks, sometimes months, distributing and holding the toxicant throughout the carrier particle and, therefore, not exposing its total quantity to the solvency action of water. Formulations prepared with low volatile solvents reduces very little in weight when stored.

Table 1.—Physico-chemical properties of some petroleum solvents used for impregnating attapulgite granules with parathion.

Solvent	Source	Flash Point (° F.)	Distillation (° F.) Init. Boil. Point	End Point	Range	Aromatics % Volume	Evaporation Time (min.)[a]
AR-35	Velsicol Corporation	< 40[b]	214	278	64		
AR-50		180[c]	390	550	160		
AR-55		200[c]	415	550	135		
AR-60		220[c]	460	560	100		
Tolu-Sol W	Shell Oil Company	< 20[b]	196	228	32	5	
Cyclo-Sol 27		35[b]	212	229	17	77	
Cyclo-Sol 37		75[b]	269	282	13	75	
Cyclo-Sol 35		90[b]	282	357	55	90	
TS-28		111[b]	319	375	56	58	
Cyclo-Sol 53W		120[b]	306	340	35	96	
Cyclo-Sol 68W		151[b]	364	418	54	98	
Aromatic Solvent 42		164[b]	383	498	115	66	
RX-4	Amoco Chem. Corporation	81[b]	282	320	38	94	4.0
RX-3		81[b]	276	360	84	76	5.3
RX-22		54[b]	368	420	52	98	19.2
AN-1		165[c]	400	494	94	98	121.0
AN-5K		190[c]	440	705	265	99	—
AN-3		220[c]	450	534	84	71	190.0
Toluol	Penola Oil Company	44[b]	229	232	3	98	
Process Naphtha 606		26[d]	206	230	24	10	
Solvent 1		< 0[d]	129	244	115	10	
Varsol High Flash 626		113[b]	324	384	60	9	
Heavy Aromatic Naphtha		136[b]	344	533	189	85	

[a] Determined at 73° F. and 61% relative humidity. Toluene = 1 min.
[b] Determined by the Tag-Closed Cup method.
[c] Determined by the Cleveland Open-Cup method. This method yields slightly higher flash point values than those determined by the TCC method.
[d] Calculated: Flash. Point in ° F. (TCC) = 0.79 (IBP)—136.

On the other hand, a highly volatile solvent evaporates quickly—within a few hours or days—depositing the toxicant near the surface, and great loss in weight was recorded for these formulations in storage.

With a short lapse of time such as studied here, the parathion molecules in the case of the high volatile solvent (AR-35) formulation distribute themselves to the inner portions of the particle and cling to adsorptive surfaces. These surfaces have already been occupied from the start with parathion molecules when a solvent of low volatility is employed. With slow evaporation of the less volatile solvents, the toxicant moves very slowly to the surface, thus availing more toxicant to the water.

Espesol Solvents.—Three solvents with high aromatic contents were obtained from Eastern States Petroleum and Chemical Corporation. Espesol 1 and Espesol 2 had 98% aromatic fractions while Espesol 5 had only 86% aromatics. The flash points of these solvents were 115° F., 146° F. and 88° F., respectively, as measured by the TCC method. Espesol 1 and Espesol 2 had higher minimum (initial boiling point) and maximum (end point) boiling points than Espesol 5, but the distillation range of the last solvent was wider than those of the other two solvents.

These three solvents with relatively similar chemical composition and close physical properties yielded similar results (fig. 3). It is apparent that both in the fresh and aged formulations Espesol 5 proved to be slightly more effective than the others. Based on the physical properties of these solvents, this difference, though not appreciable, was expected. These solvents are moderately efficient as far as speed of release of parathion is concerned.

Standard Oil of California Solvents.—The twelve solvents of Standard Oil Company of California varied a great deal in their aromatic, paraffinic, and naphthenic contents. Some of these solvents, such as Socal solvents 2, 3, and 51L, had 90% or more aromatic content. Socal 170L had 84% aromatic content and the next highest in aromatic contents were Socal solvents 305L and 230L. The last 2 solvents (see fig. 4) were considerably lower (70% and 69%) in their aromatic content with a corresponding increase in paraffinic and naphthenic fractions.

Most of the Standard solvents were characterized by low-to-moderate flash points and low initial boiling points. The distillation ranges were relatively narrow except for Thinner No. 425 which had a range from initial boiling point to maximum boiling point of 137° F. Thinner No. 221 was characterized by a very low flash point (14° F.), which makes use of this solvent hazardous in commercial production of granular materials. Chevron Light Solvent and Socal 51L each had a flash point of 40° F. as determined by TCC method, The remaining solvents had flash points closer to that of xylene.

Here again the use of solvents with low flash points, low initial boiling points, and narrow distillation ranges resulted in slightly better release of the toxicant (fig. 4). Of the fresh formulations, Socal 305L, Socal 170L, and Socal 3 released smaller quantities of parathion in the water than Thinner 425, Thinner 221, and Socal 51L. After 12 weeks of aging, the more volatile solvents, Socal 51L and Thinner 425, slowed down in their speed of re-

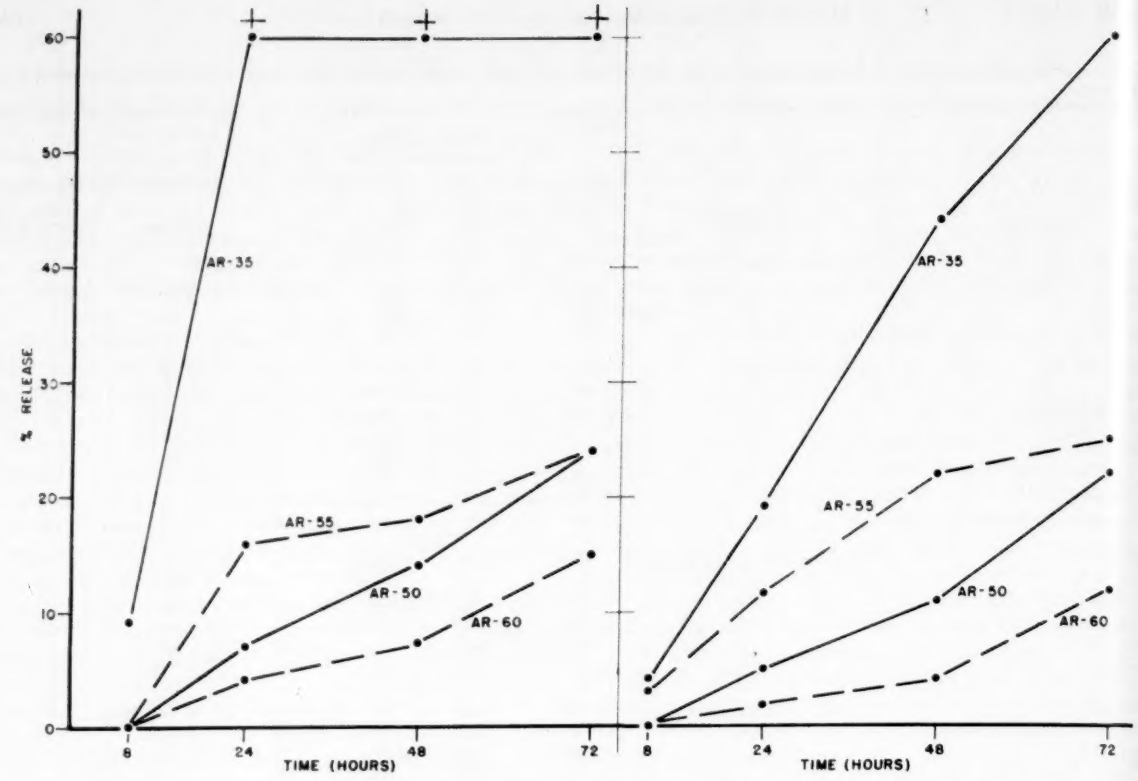

FIG. 2.—Release into water of parathion from granular formulations prepared with Velsicol solvents. (Left: fresh formulations; right: formulations aged for 18 weeks.)

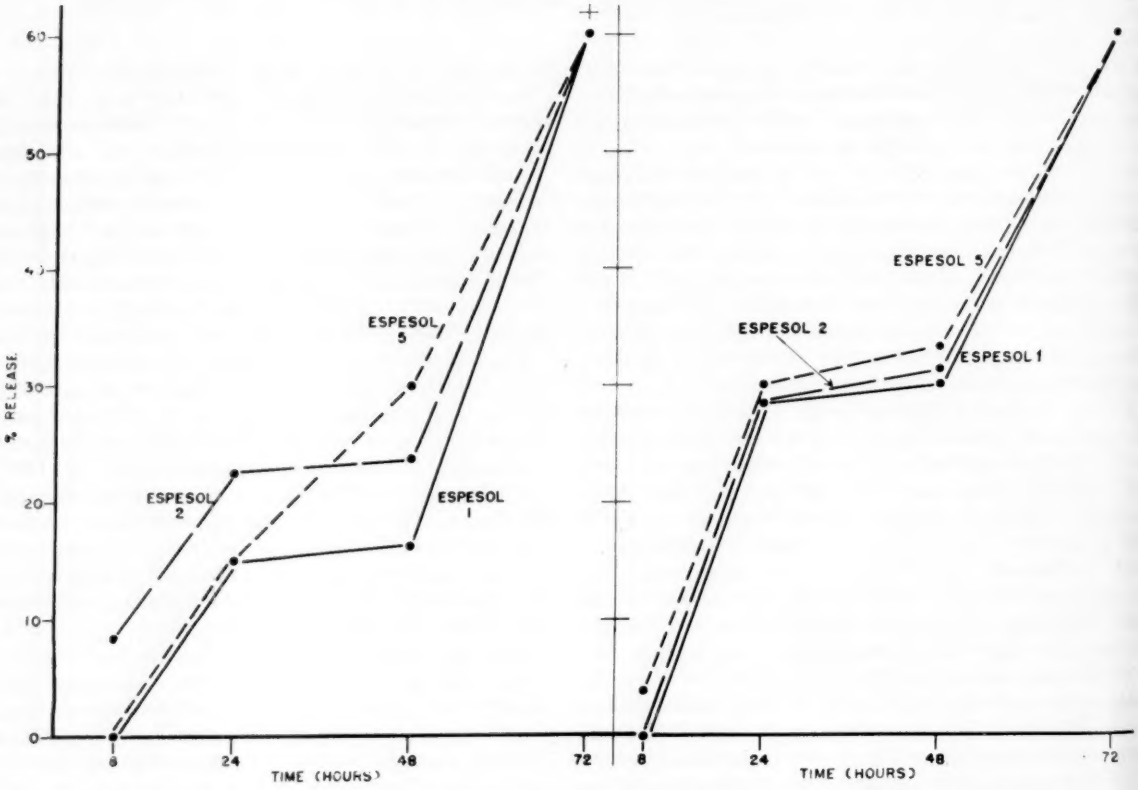

FIG. 3.—Release into water of parathion from granular formulations prepared with Espesol solvents. (Left: fresh; right: 21 weeks old formulations.)

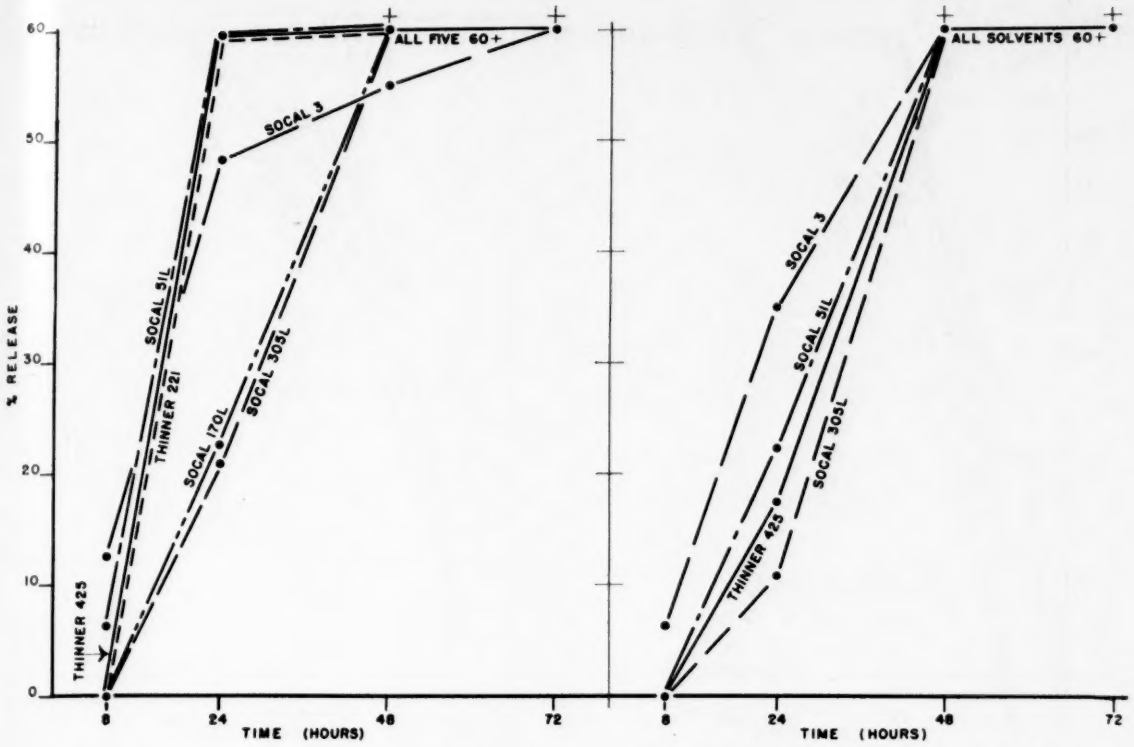

FIG. 4.—Release into water of parathion from granular formulations prepared with Standard Oil Company of California solvents. (Left: fresh; right: 12 weeks old formulations.)

lease of parathion and fell more or less in line with the less volatile solvents Socal 305L and Socal 3 which also, on aging, slowed down slightly in their speed of release.

A similar pattern was observed for a second group of Standard solvents (fig. 5). When tested 1 day after preparation, Chevron Light Solvent, Socal 93L, Socal 98L, and Socal 230L gave slightly faster release of the toxicant than Socal 2 and Socal 306L. The last two solvents had higher flash points, more moderate initial boiling points, and wider distillation ranges than the other solvents in the group. After they had been aged for 11 weeks, the less volatile solvents Socal 306L and Socal 2 followed almost the same pattern that they had prior to aging. The more volatile solvents, after aging of the formulation, dropped slightly in their ability to effect a rapid and high release of parathion. The differences of the physical properties among Standard Oil solvents are not great enough to yield marked differences among the granular formulations prepared with these solvents by the testing procedure used.

Amsco Solvents.—Six representative solvents of a total of well over 40 available from American Mineral Spirits Company were evaluated. Two of them, Lactol Spirits and Solvent A-80, presented potential fire hazards because of their very low flash points of 22° F. and 18° F., respectively. They also showed narrow ranges of distillation and the lowest initial boiling points in this group of solvents. Lactol Spirits had 62% naphthenes and 31% paraffins by volume; Solvent A-80 had 63% aromatics, 25% naphthenes and 12% paraffins. Solvent B had a flash point of 80° F., moderate initial boiling point, narrow range of distillation, and had 87% aromatics by volume. The remaining solvents, Naphthol Spirits, 140-H Solvent, and Solvent G, had moderately high flash points (102° F., 143° F. and 146° F., respectively, as measured by the TCC method), moderate initial boiling points, and fairly narrow ranges of distillation. Solvent G was high in aromatic content (98%), Naphthol Spirits high in paraffin content (83%), and 140-H Solvent high in paraffins and naphthenes.

In evaluating the fresh formulations it is obvious that the highly volatile solvents, Lactol Spirits and Solvent A-80, gave more rapid and higher release of parathion than the other solvents (fig. 6). The remaining four solvents follow each other rather closely with Naphthol Spirits and Solvent G manifesting more efficiency than 140-H Solvent and Solvent B.

After aging, the most volatile solvent, Solvent A-80, showed a decreased speed of release of the toxicant, while the more volatile solvents dropped slightly in effecting rapid release although holding to the same relative positions as prior to aging. This trend was also observed in the tests with the Standard solvents.

Shell Oil Company Solvents.—Shell petroleum solvents investigated for their use in granular formulations and their physical and chemical properties are presented in table 1. These are representative solvents from among the more than 30 aliphatic and aromatic solvents commercially available from Shell Oil Company on the West Coast.

Cyclo-Sol 27 and Tolu-Sol W having the lowest flash points and initial boiling points and narrow distillation

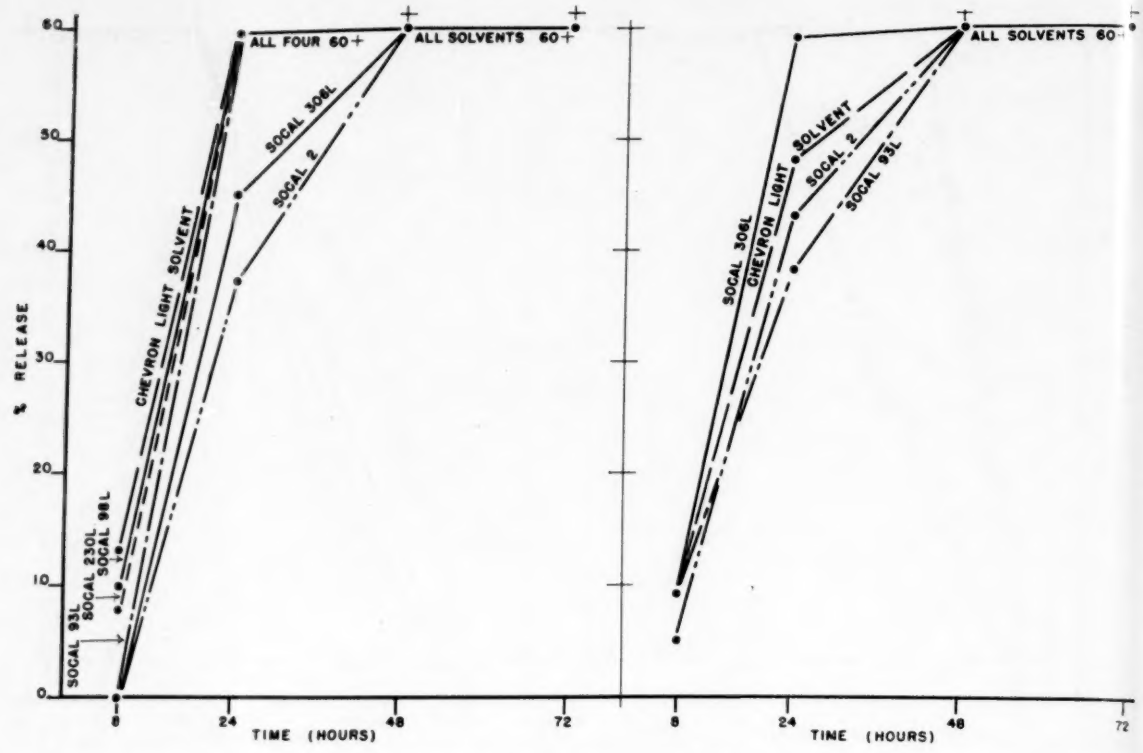

FIG. 5.—Release into water of parathion from granular formulations prepared with Standard Oil Company of California solvents. (Left: fresh; right: 11 weeks old formulations.)

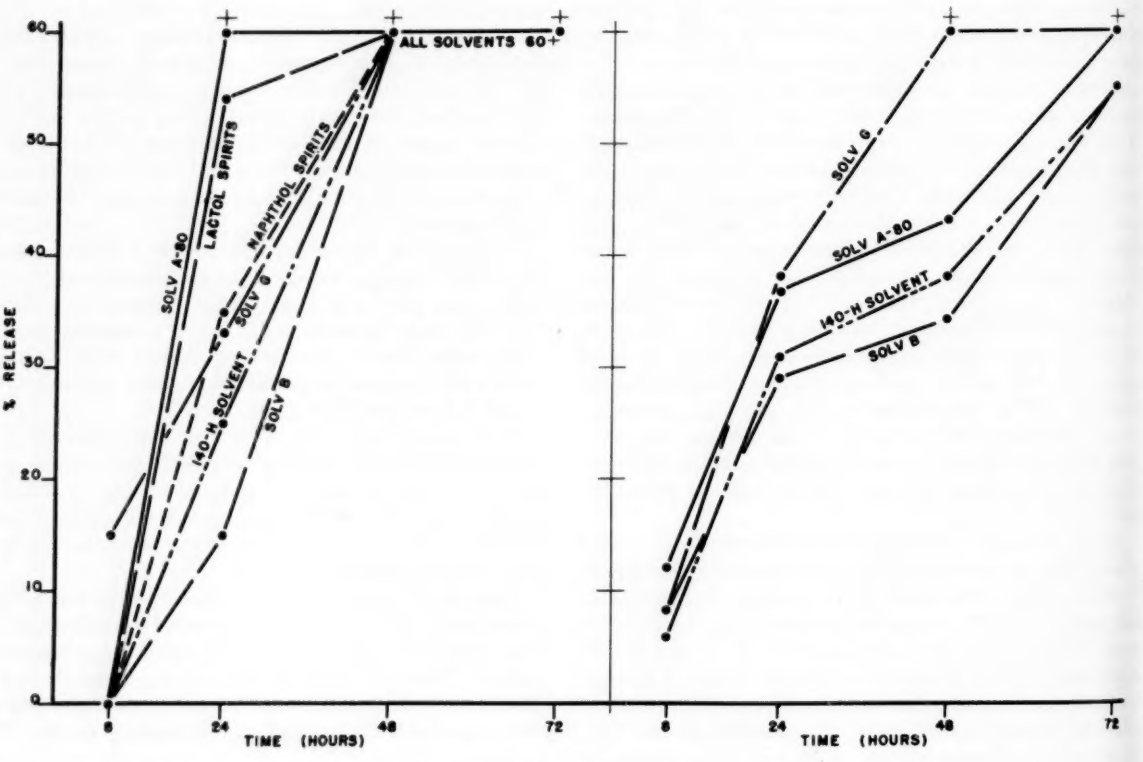

FIG. 6.—Release into water of parathion from granular formulations prepared with Amsco solvents. (Left: fresh; right: 10 weeks old formulations.)

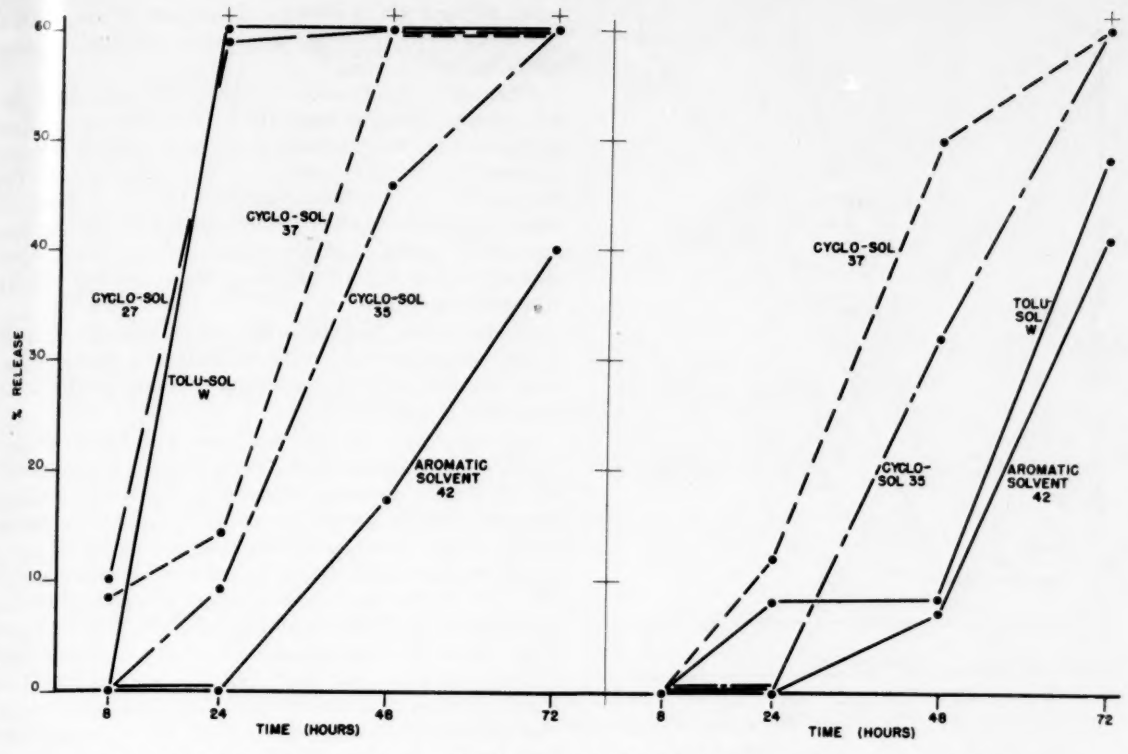

FIG. 7.—Release into water of parathion from granular formulations prepared with Shell solvents. (Left: fresh; right: 16 weeks old formulations.)

ranges, yielded greater release of parathion in the fresh formulations than the other solvents in this group (fig. 7). Cyclo-Sol 37 and 35, with intermediate properties, resulted in slower release of the toxicant in these formulations. Aromatic Solvent 42 with high flash point, high initial boiling point, and wide distillation range gave gradual but much slower release of the toxicant.

After aging for 16 weeks, the most volatile solvent, Tolu-Sol W, decreased markedly in the release of parathion from the granular formulation. Solvents with intermediate volatility produced more or less the same results as their fresh formulations but with some decrease in the rate of release. There was more decrease in the 48-hour level of release of the toxicant for the aged formulation of Solvent 42 than for its fresh formulation and this drop in release could be attributed to experimental variation. Excluding biological variations, it seems that the moderately volatile solvents result in the same pattern of release when either fresh or aged for 3 to 4 months.

Three other Shell solvents—TS-28, Cyclo-Sol 53W, and Cyclo-Sol 68W—were evaluated. The flash points, initial boiling points, and distillation ranges of TS-28 and Cyclo-Sol 53W were lower than those of Cyclo-Sol 68W (see table 2). The first two solvents gave faster and higher release of the toxicant than the last (fig. 8). These solvents were not tested after aging.

Panasol Solvents.—The role of two series of Amoco aromatic solvents in the efficiency of granulated parathion was investigated. These solvents with some of their chemical and physical properties are presented in table 1.

The speed and magnitude of release of the toxicant from the granular formulations prepared with these solvents were found to be markedly different for the two series of solvents. The RX series of solvents released the toxicant much faster than the AN series when the formulations were tested fresh (fig. 9). In the former series, RX-22 gave slightly slower release than the other two in this series. This trend was expected since although RX-22 has a low flash point, it has a much higher initial boiling point than the other two RX solvents. In the AN series of solvents, AN-3 has the highest flash point and the highest initial boiling point. Therefore, it is expected to give slower and lower release of the toxicant. The rate and magnitude of release using the two series of solvents are also observed to be inversely related to the evaporation time of the sol-

Table 2.—Some petroleum solvents with REI values and efficiency in releasing parathion from freshly prepared granules.

SOLVENT	REI VALUE	APPROXIMATE PARATHION RELEASE AT 24 HOURS (%)
AR-60	101.20	4
Solvent 42	72.23	3
AN-1	62.04	25
Solvent G	29.59	33
Socal 305L	17.95	21
RX-22	10.33	50
AR-35	5.48	60+
Solvent A-80	1.36	60+
Cyclo-Sol 27	1.26	60+
Chevron Light Solvent	3.43	60+

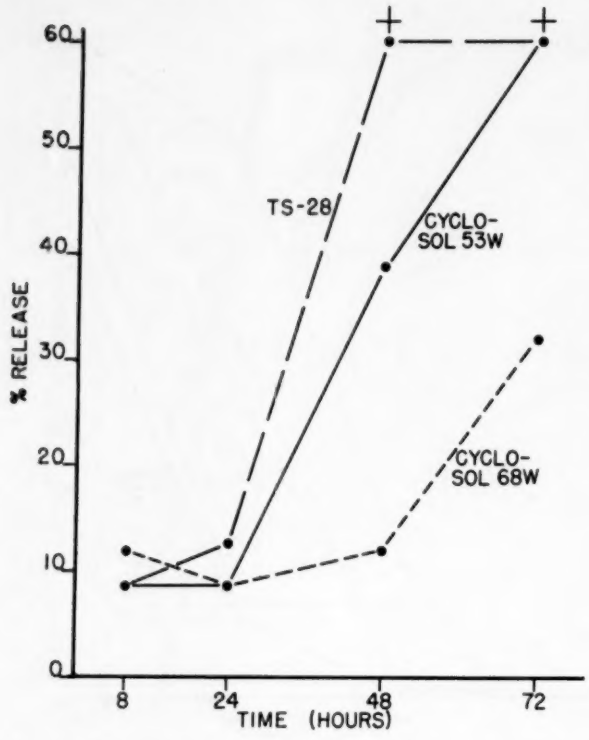

FIG. 8.—Release into water of parathion from fresh granular formulations prepared with Shell solvents.

vents. Solvent AN-3, with the lowest rate of evaporation of all the solvents in this experiment, resulted in slow-releasing formulations.

After aging, the formulations prepared with the Panasol solvents behaved similarly to the fresh ones. Here again the aged formulations of the more volatile solvents released the toxicant slower than the fresh materials. The less volatile solvent formulations yielded more rapid release than when they were tested fresh. After having been aged for 13 weeks, the more volatile solvent formulations showed higher overall efficiency than the less volatile solvent formulations.

Penola Solvents.—Five of the Penola aromatic and aliphatic petroleum solvents were evaluated. Some important physico-chemical properties of these products are presented in table 1.

An examination of the flash points of these solvents would suggest that Solvent 1 (with a very low flash point) in this series is the most efficient one for use in granular formulations. However, the wide range of distillation (115° F.) of this solvent determined that its efficiency would be lower than that of Toluol and Process Naphtha 606 (with higher flash points but with narrower distillation ranges) and approximately equal to that of Varsol High Flash 626 (fig. 10). This last solvent has a much higher flash point, but its distillation interval (60° F.) is narrower than that of Solvent 1. This demonstrates that the range of distillation of a solvent plays an important role in the efficiency of granulated insecticides. Heavy Aromatic Naphtha, with high flash point, high initial

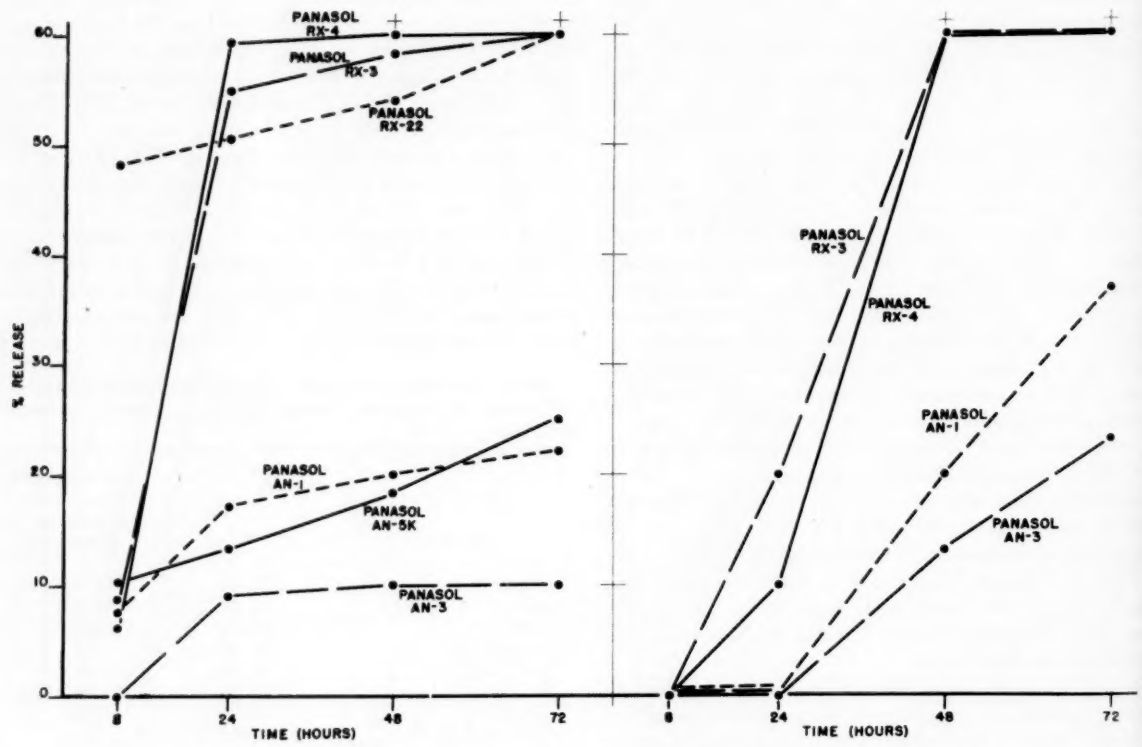

FIG. 9.—Release into water of parathion from granular formulations prepared with Panasol solvents. (Left: fresh; right: 13 weeks old formulations.)

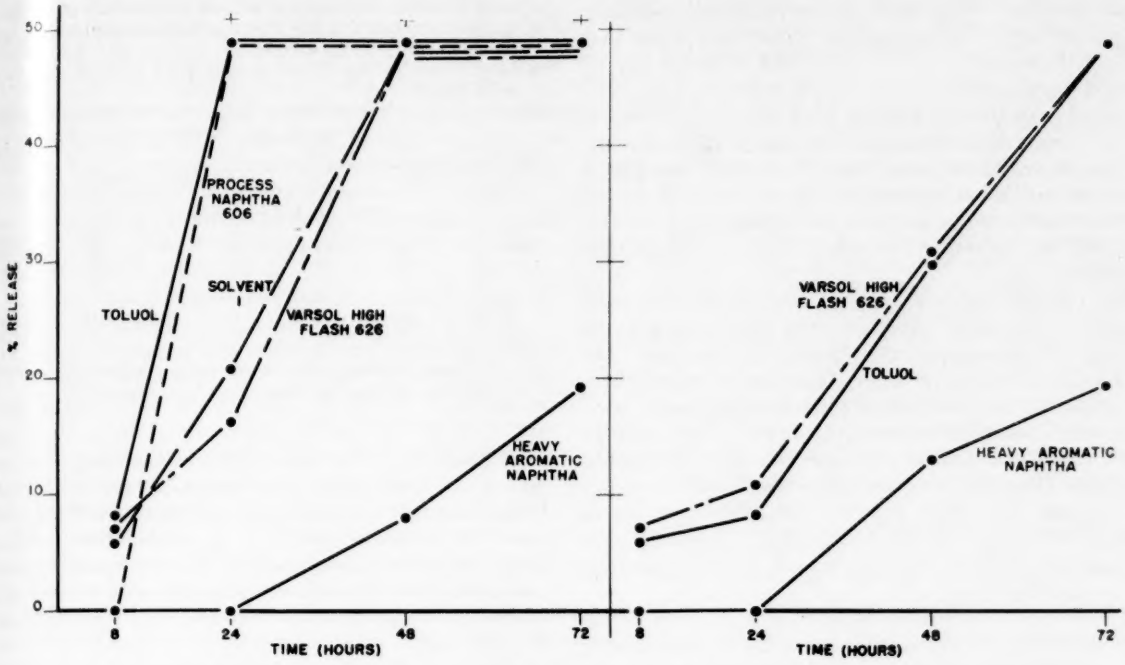

FIG. 10.—Release into water of parathion from granular formulations prepared with Penola solvents. (Left: fresh; right: 16 weeks old formulations.)

boiling point, and wide range of distillation, turned out to be a slow-releasing solvent.

After 16 weeks of aging, the formulation prepared with Toluol released the toxicant slower than when it was fresh. Varsol High Flash 626 formulation released a decreased quantity of toxicant on aging, but the less volatile solvent Heavy Aromatic Naphtha followed practically the same pattern both when fresh and aged but with somewhat higher release in the latter case.

DISCUSSION.—*Physical Properties and Solvent Efficiency.*—From the evaluation of freshly prepared granular formulations made with various petroleum solvents, it was noted that the behavior and efficiency of most of the solvents in each series could be explained in terms of specific physical properties of the solvents. An obvious relationship between the initial boiling point, maximum boiling point, distillation range, and flash point of these solvents and their efficiency in granular formulations was noted. In the case of the Panasol solvents, the efficiency was observed to be inversely related to the evaporation time, and the evaporation time of a solvent, of course, is related to the distillation pattern of that solvent. These properties are determined, in turn, by the chemical structure and composition of these solvents. The flash point is not a true physical property, but it is an important aid in predicting the role of a solvent in granular formulations. The bulk of the petroleum solvents consists of mixtures of a variety of compounds encompassing a wide range of chemical and physical properties. However, in the commercial production of petroleum solvents certain specifications are met and the products are known to have (with slight variation) typical physical properties and known chemical composition in terms of classes or types of compounds.

Flash Point and Distillation Pattern Affecting Efficiency of Petroleum Solvents.—The efficiency of a majority of the 44 petroleum solvents evaluated in fresh granular formulations was noted to be determined by their flash points, distillation curve patterns and evaporation time. An attempt was made to correlate the efficiency of petroleum solvents with some of the other physical and chemical properties, but such a correlation was not obvious. Properties such as viscosity, specific gravity, aniline values, Kauri Butanol values, and aromatic or paraffinic contents did not directly yield any clues as to the role that these properties may play in affecting the release of toxicants from granules.

The speed and magnitude of release of parathion from 30/60 mesh AA-LVM attapulgite granules were found to be inversely proportional to the flash point, initial boiling point, maximum boiling point, and the range of distillation of the various solvents. For the Panasol solvents, whose evaporation rates were known, the rate and magnitude of release were observed to be inversely related to the evaporation time. Unfortunately, information of this type is not available for all the solvents.

The initial boiling point and the flash point of the solvents are roughly interrelated. A solvent with a high flash point, high initial boiling point and wide distillation range will generally release the toxicant slower than one that has lower values for these properties. To facilitate the selection of a more efficient group of solvents (resulting in faster release) the following formula has been worked out for obtaining a relative efficiency index:

$$REI = FP \times IBP\ (EP\text{-}IBP) \times 10^{-5}$$

Where REI is the relative efficiency index, FP is the flash point, IBP is the initial boiling point and EP is the end

point. For practical purposes, the petroleum solvents may be grouped into three categories. It has been noted that 80% of the solvents tested having REI values of 1 to 20 yielded rapid release of parathion. Solvents with REI values of more than 20 and less than 70 mostly resulted in slower release of the toxicant. Very low volatile solvents having REI values of more than 70 generally resulted in formulations which released the toxicants very slowly. Such solvents are not desirable for impregnating the carrier with the toxicant where rapid release of the toxicant is desired.

The formula does not apply to solvents that are pure chemically. These solvents have either fixed boiling points or very narrow ranges of a few degrees of distillation. The REI values of such solvents will be either zero or very low. In predicting the behavior of pure solvents, vapor pressure values play an important role. Preliminary tests with a few chlorinated hydrocarbon solvents have established this fact. The vapor pressure values are known for only a few of these chemically pure solvents (Raikes & Bowen 1928, Weissberger & Proskauer 1955). Also, solvents having flash points below 0° F. provide another exception to the formula.

The formula-determined REI values of a few of the petroleum solvents are given in table 2. The approximate per cent releases of parathion obtained from granular formulations impregnated with these solvents are also included. A gross correlation between the REI values and the efficiency of the solvents as determined by the release of parathion is obvious. Generally, an increase in release of parathion is associated with a decrease in the REI values of the solvents. However, it should be remembered that the present procedure of obtaining experimental evidence is not intended for determining small differences in the performance of the various solvents as dictated by the REI values. The REI values, however, separate those solvents from each other when these have a wide variation in their distillation patterns. Solvents having closer distillation characteristics are not usually separated by this approach.

Experimental evidence gained thus far tells us that solvents with REI values in the range of 1 to 10 are generally more efficient (releasing parathion faster) than those with intermediate REI values. Solvents having the intermediate REI values are again more efficient than those with much higher REI values (above 70 or 80). These limits of REI values are arbitrary and have overlapping values but are helpful in selecting an efficient solvent from among the more than 500 brands of petroleum solvents available commercially. Among the 44 solvents investigated, no solvent with high or moderate efficiency had REI values of more than 80. Solvents with REI values of less than one are extremely volatile and constitute fire hazards.

As stated earlier, the REI value of a solvent is a function of the flash point, initial boiling point, and distillation range of that solvent. In addition to the REI value, a comparison of the values of these physical characteristics as stated above also permits prediction of the behavior of a solvent in determining the speed of release of parathion from its granules. An overall average of values for these characteristics of the 44 petroleum solvents suggests that these characteristics influence the behavior of the solvents in the performance of granular formulations of insecticides (table 3). As the flash point, initial boiling point, end point, and distillation range increased, the REI values correspondingly increased with a resultant decrease in the speed of release of parathion. A reverse trend in these properties of the solvents will result in increased release of parathion from the granules prepared with the solvents. These trends are obvious both from the fresh and aged formulations (see table 3).

Table 3.—The efficiency of petroleum solvents in granular formulations related to the average flash points, initial boiling points, end points, distillation ranges and REI values, as determined by the extent of parathion release into water after 24 hours.

Parathion Release (%)	No. Solvents Tested	Average Flash Point (° F.)	Average Distillation (° F.)			Average REI Value
			IBP	End Point	Range	
Fresh formulations						
0– 30	24	128	337	414	64	44.56
30– 60	10	91	279	357	59	21.30
60–100	12	58	233	291	58	11.49
Aged formulations[a]						
0– 30	24	112	314	385	74	38.67
30– 60	8	96	282	339	58	16.83
60–100	0	—	—	—	—	—

[a] Formulations aged between 10 and 21 weeks at room temperature.

Aging.—Aging of granular formulations prepared with petroleum solvents produced an interesting trend. The highly volatile solvent formulations, after aging from 10 to 21 weeks, resulted in lower release of parathion than the fresh formulations. Conversely, the aged formulations prepared with low-volatile solvents increased slightly in releasing parathion from the granular formulations. The intermediate range solvents did not produce any marked difference between their fresh and aged formulations.

Mechanism of Action of Solvents.—It has been determined that the use of solvents for impregnating inert carriers with parathion increased the speed of release of the toxicant into the surrounding water. This action was probably owing to the uniform coverage of the inert particles with the active material and a better distribution of the toxicant on the inert surfaces. Carriers impregnated with liquid toxicants without the use of an efficient solvent have yielded erratic results.

In the process of impregnation of granular particles with a toxicant, the toxicant may be deposited either near the surface or carried through the entire body of the granular particles depending on the type of solvent and carrier particles. Highly volatile solvents will evaporate quickly from the surface of a granule and carry the toxicant in the liquid film that moves outward in the capillaries of the particles. The rate of movement of the solution film will depend on the vapor pressure and distillation and evaporation characteristics of a particular solvent and the type of carrier. With complete evaporation of the solvent, the magnitude and rate of dissolution of a toxicant in water will depend on the adsorption forces inherent in the carrier and the solubility of the toxicant in water. Parathion is soluble in water up to 20 p.p.m. and 100% releases have been obtained from some of its granular formulations prepared with highly volatile solvents and applied to water at 5 p.p.m. concentration of actual toxicant.

After evaporation of the solvent and deposition of the toxicant at the surface, a reverse process is more likely to

ensue. In this process the toxicant molecules possibly move as a liquid film or diffuse as vapors into the inner portion of the carrier particles. The speed of this process from the surface toward the center of the particle will likely depend on the vapor pressure and other properties of the toxicant, and the adsorption characteristics of the carrier. For parathion this affinity between the toxicant molecules and the adsorptive surfaces seems to be small, as suggested by the study of the aged formulations, and the solvency power of water can remove all of the parathion from the clay particles.

Low-volatile solvents, on the other hand, evaporate very slowly and only the low-boiling fractions are expected to evaporate in a relatively short period of time. Therefore, the toxicant is held in solution form in the carrier particle. As the low-boiling fractions evaporate, the quantity of the toxicant deposited at the surface is gradually increased. It is probably because of this phenomenon that low-volatile solvent formulations released slightly more parathion on aging.

The theory of the distribution, retention, and movement of toxicant molecules in the granular particles as regulated by the type of solvent explains the mechanism of action of solvents in the behavior of granular formulations. However, it is believed that this explanation does not present the complete picture. Other possible mechanisms of action of solvents should not be overlooked in attempting to understand completely the behavior of granulated insecticides. The data obtained here with parathion, on only one type of carrier, suggest certain definite trends but should be applied cautiously to other types of granulated insecticides. It is possible that other combinations of carriers and toxicants may yield results different from those reported here for the various petroleum solvents.

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Effectiveness of Various Formulations of Five Insecticides on Insects Infesting Douglas-Fir Logs[1,2]

J. A. RUDINSKY,[3] L. C. TERRIERE,[3] and D. G. ALLEN[4]

ABSTRACT

Application of endrin, lindane, Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide), Sevin® (1-naphthyl methylcarbamate), and heptachlor in solution, suspension and emulsion to the bark of freshly cut Douglas-fir logs (*Pseudotsuga menziesii* (Mirb.) Franco) provided very good protection from infestation by the Douglas-fir beetle (*Dendroctonus pseudotsugae* Hopk.) and other bark and wood boring insects. Emulsions and solutions, however, were more effective than suspensions. Field bioassays and bark analyses of the treated logs 10 weeks after application indicated that much of the insecticides penetrated the bark, particularly in the solution and emulsion forms.

Wood fiber surfaces, treated with emulsions and suspensions of the five insecticides, were used for studying residual toxicity under constant laboratory conditions for a 7-week period. Wettable powders of Thiodan, endrin, and Sevin show gradual decline in residual toxicity, whereas a rapid decrease was shown by heptachlor and lindane. The emulsions exhibited a parallel but lower toxicity in all cases.

Green logs left in the forest during periods of insect activity are usually degraded by harmful insects and fungi. In recent years, there has been an intensified search for insecticides with lasting residual properties to use in

[1] Technical paper No. 1313, Oregon Agricultural Experiment Station. This paper presents the results obtained in a research project sponsored jointly by the Oregon State College Agricultural Experiment Station, the Oregon Forest Lands Research Center and the Northwest Forest Pest Action Council. It was supported by the Regenstein Foundation, the Foundation for American

the protection of such logs. DDT and BHC, in various formulations, have been commonly used. According to Becker (1947) and Liming *et al.* (1948), emulsions prepared from DDT emulsifiable concentrates were more effective against elm bark beetles and had longer residual life than DDT suspensions. Blais (1953) found that DDT in oil solution was more effective against *Monochamus* spp. on various coniferous logs than was a wettable powder suspension. With lindane and BHC, however, little difference in control resulted whether the formulation was an oil solution, a wettable powder, or an emulsifiable concentrate (Kowal 1958, Kinghorn & Webb 1950, Becker 1955, 1959, Hetrick & Moses 1953, Lyon 1959, and others). Hetrick (1957) showed that Sevin in emulsion gave good protection for about 6 weeks against pine bark beetles. Similar results with Sevin® (1-naphthyl methylcarbamate) in wettable powder form were obtained against the Douglas-fir beetle, *Dendroctonus pseudotsugae* Hopk., and other wood borers by Allen & Rudinsky (1959), who also used Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide) and lindane in wettable powder form with excellent results.

In the Pacific region, initial infestation by the Douglas-fir beetle and the ambrosia beetle (*Gnathotrichus sulcatus* LeConte) may occur as early as the end of March or April. After early warm temperatures, adverse weather may set in, occasionally lasting until late July. The residues of insecticides deposited on log surfaces may persist in such weather to different degrees according to the pesticide and formulation used; thus, it is worthwhile to evaluate different formulations of a pesticide with these factors in mind. The objective of this study was therefore to make a field comparison of the relative effectiveness of wettable powders, emulsifiable concentrates, and solutions against the Douglas-fir beetle and other insects attacking freshly cut Douglas-fir logs, *Pseudotsuga menziesii* (Mirb.) Franco. The insecticides used were Thiodan, Sevin, lindane, endrin, and heptachlor.

MATERIALS AND METHODS.—*Field Tests.*—Ten Douglas-firs in a 160-year-old stand were cut on April 20, 1959, in the Mary's Peak area (Siuslaw National Forest) at an elevation of 1,200 feet. The felled logs, which were left intact to reduce drying, ranged from 19 to 32 inches in diameter. The six logs selected for the insecticide tests were divided into sections, each about 5 feet long with a surface area of 25 square feet, and the sections were separated by 1-foot buffer zones to reduce cross-contamination during spraying. Each treatment was replicated four times, providing a total of 100 square feet of surface per formulation. The effect of variations in the bark thickness and texture from butt to crown was reduced by alternating the order of the materials to be sprayed on each tree. The four remaining trees as well as the crowns of the treated trees were used as untreated controls. Late in May, by which time the control trees had been heavily attacked, two additional trees were cut. They provided data on insect activity after the major attack and until the third week of July when the attacks had ceased.

The sprays were applied at the rate of 200 mgs. active ingredient per square foot with Thiodan, endrin, and lindane, and 400 mgs. per square foot with heptachlor and Sevin. The following formulations were used in preparing the dilute sprays: Sevin wettable, 50%, emulsifiable, 1.0 lb. per gallon, technical, 95%; endrin wettable, 25%, emulsifiable, 1.6 lbs. per gallon, technical, 95%; lindane wettable 25%, emulsifiable, 1.6 lbs. per gallon, technical, 95%; heptachlor wettable, 25%, emulsifiable, 2.0 lbs. per gallon, technical, 72%; Thiodan wettable, 25%, emulsifiable, 2.0 lbs. per gallon, technical, 98%. The treatments also included two controls, one untreated and one sprayed with the solvent used in preparing the solutions. Air temperature, relative humidity, and rainfall were recorded throughout the experiment.

The emulsifiable and wettable powder formulations were applied in about 2 gallons of water and the solution in 1 gallon of solvent using small compressed-air sprayers. The solvent used was a 4:6 mixture of base oil C (refined kerosene) and Velsicol AR 50, except that Sevin required the addition of acetone to provide a mixture of 1:2:3, acetone, base oil C, and Velsicol AR 50.

Treated and check trees were examined weekly, and new entrance holes were marked with different signs and adhesive paint each week. Thus, it was possible at the time of final examination and debarking on September 2 (20 weeks after spraying) to compare each attack seen on the outside bark with its actual penetration and brood development under the bark. Three types of Douglas-fir beetle invasions were recorded: first, "attempted" invasion in which the beetles died before they penetrated to the cambium and therefore did not introduce any blue stain; second, "abortive" invasion in which the beetles penetrated into the cambium, introduced blue stain, and even started to excavate galleries but did not produce any progeny; third, "successful" invasion in which the beetles established a brood. In the latter kind of invasion it was also necessary to determine whether the insect reached adult stage or died in an early instar, since early death could have resulted from penetration of insecticide into the inner bark. On final examination ambrosia beetle invasions were counted, and the per cent of the area invaded by round-headed borers and by stain and rot fungi was estimated.

At the end of the 10th week after spraying, bark samples were taken for analysis to determine the insecticide content. By use of a sharp steel pipe $1\frac{3}{4}$ inches in diameter, two samples were cut from each of the replicates, one from the top and one from the side of the log to provide a total of 19.2 square inches of sprayed bark for each analysis. The eight sample cores from each treatment were stored under refrigeration for 6 months pending analysis. Only lindane, heptachlor, and endrin were analyzed. It was impossible to take lateral subsamples through the cores to determine the depth of penetration of the various formulations because some of the cores had become broken in storage. Instead, the samples were ground and extracted with solvent to determine the total amount of pesticide present. Lindane and heptachlor were analyzed by a mosquito larvae bioassay, and endrin by the specific

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phenyl azide method of Bann *et al.* (1958).

At the time the bark samples were taken for insecticide analysis, an attempt was made to assess the relative effect of toxicants remaining on the surface of the bark. This was done by field bioassays using laboratory-reared Douglas-fir beetles (Ryan 1959). Ten beetles were confined to a portion of the treated surface using small cans (four cans per assay). After 40 minutes of exposure, the beetles were transferred to petri dishes and returned to the laboratory for 72-hour mortality counts (table 2).

LABORATORY TESTS.—Further evaluation of the relative toxicity of various formulations was made in the laboratory using techniques described by Rudinsky & Terriere (1959). Wettable powder and emulsifiable formulations of the five insecticides used in the field tests were applied to Firtex blocks at rates of 75 mgs. per square foot (Thiodan, lindane, and endrin) and 150 mgs. per square foot (heptachlor and Sevin). Beetles were exposed to the blocks at 0, 4, and 7 weeks after application, using three fresh blocks for each exposure. Until exposure, the treated blocks were held at 78° F. in subdued light.

RESULTS AND DISCUSSION.—*Field Studies.*—Table 1, summarizing the results of the field tests, shows the total number of invasions of the treated logs by Douglas-fir beetles and ambrosia beetles, and an estimate of the per cent of the area invaded by buprestids and blue stain. Comparison of the total number of successful invasions of the untreated controls with those occurring on the variously treated logs shows that during the 20 weeks of the tests the treatments were more than 98% effective. Except for Sevin, no invasions occurred during the first 8 weeks after treatment. It was noted that the larvae resulting from some of the early invasions died in the first and second instar, suggesting that the toxicant may have penetrated the inner bark in these cases. The log sections treated with the solvent mixture were relatively free from attack although some occurred during the whole active season. This degree of control may have been caused by repellency since no toxic effects appeared in developing larvae. Such repellency may explain in part the greater effectiveness of the solutions over the emulsions.

There were only occasional ambrosia beetle attacks on treated logs and only moderate attacks (4.1 per square foot) on control trees. This was owing to reduced ambrosia beetle activity during the test, as the peak of activity had taken place almost 3 weeks prior to cutting and spraying of the trees. An interesting and unexpected result was the heavy concentration of *Melanophila drummondi* (Kby.) on logs treated with Sevin. Since this effect was noted in all of the Sevin formulations, it seems likely that the toxicant itself was the cause of the phenomenon. It is also interesting to note that these invasions, which occurred in June, were not accompanied by the characteristic blue stain infections.

The blue stain associated with the entry of Douglas-fir beetles was minimal. There were no white mycelial mats of decay fungi on treated logs, whereas on untreated logs about 25% of the area was covered with mats, and pene-

Table 1.—The protection of Douglas-fir logs from insect invasion by three formulations of endrin, Thiodan, lindane, Sevin and heptachlor during a 20-week period.

TREATMENT (MG. ACTIVE INGREDIENT /SQ. FT.)	NUMBER INSECT INVASIONS PER 100 SQ. FT.					
	Douglas-Fir Beetle			Ambrosia Beetle	Estimated Per Cent Invasion by	
	No. Successful and Date[a]	Abortive	Attempted	Successful	Buprestids	Blue Stain
Endrin, 200						
Solution	None	8	5	2	None	0.5
Suspension	1 6/16	26	20	5	0.5	2.0
Emulsion	None	12	16	4	None	0.5
Lindane, 200						
Solution	None	2	None	None	None	Trace
Suspension	2 6/23	15	9	4	None	1.0
Emulsion	1[b] 6/16	5	10	2	None	1.0
Thiodan, 200						
Solution	1[b] 6/16	9	4	2	0.5	1.5
Suspension	1 6/30	28	15	9	1.0	2.5
Emulsion	2 6/23	17	10	5	3.0	1.5
Sevin, 400						
Solution	1[b] 5/19	6	4	4	55.0	1.0
Suspension	4 5/26	22	8	10	60.0	3.0
Emulsion	3 5/28	14	8	10	55.0	2.0
Heptachlor, 400						
Solution	2[b] 6/16	12	12	7	None	1.0
Suspension	3 6/23	10	10	11	2.0	1.5
Emulsion	None	8	9	12	None	0.5
Solvent	11 4/28	38	28	22	2.0	4.5
None	312 4/20	52	None	412	6.5	95.0

[a] Date of first successful invasion; toxicants applied April 20.
[b] Larvae died in first and second instars.

Table 2.—Results of field bioassay of logs 10 weeks after treatment using laboratory-reared Douglas-fir beetle.

Insecticide	Per Cent Mortality by		
	Suspension	Emulsion	Solution
Endrin	7.5	10.0	7.0
Lindane	7.0	9.0	9.5
Thiodan	20.0	18.5	14.0
Sevin	0.0	7.0	5.0
Heptachlor	12.0	10.0	13.0
Control	0.0	0.0	0.0

tration into the sapwood was already in progress.

The results of the field bioassays of the treated logs (table 2) indicate that after 10 weeks of exposure to the weather some toxicant remained on the surface. The low mortality shown in these tests compared with the results of the bark analyses from the heptachlor, endrin, and lindane treatments indicates that much of the applied dose penetrated the bark. The approximate percentages of the original deposits remaining at the end of the 10th week are as follows: lindane solution, 50, suspension, 20, emulsion, 22; endrin solution, 30, suspension, 19, emulsion. 39; and heptachlor solution, 70, suspension, 50, emulsion, 55. The results with heptachlor were surprising in view of its known residual life on other surfaces. A paper chromatographic examination of the heptachlor extracts revealed that a large percentage of the toxicant present was actually heptachlor epoxide, a byproduct of heptachlor degradation. Since this material is more toxic to mosquito larvae than the parent compound, the bioassay results, based on the toxicity of heptachlor, would tend to be high. Although the relative amount of heptachlor in the three formulations would not be affected by this error, the percentage of the original dose present at the time of analysis would actually be somewhat less than that indicated.

Laboratory Tests.—The mortality resulting from a 5-minute exposure of the beetles to the sprayed blocks kept in the laboratory during the 7-week period is shown in Table 3. Wettable powders of Thiodan, endrin, and Sevin show gradual decline in residual toxicity during the first weeks, whereas a rapid decrease is shown by heptachlor and lindane. The relative initial toxicities and the pattern of residual decline are similar to those obtained in previous tests (Rudinsky & Terriere 1959) except for the longer residual life shown in the former tests. Since the insects used in the present tests were laboratory-reared, while those used in the former studies were field-collected, it was thought possible that a difference in susceptibility may have led to the lower toxic effects. However, in comparing insects from the two sources no such differences were found.

As the data in table 3 indicate, the emulsions exhibited a parallel but lower toxicity in all cases. This is probably owing to the more rapid and extensive penetration of the wood fiber blocks by this formulation.

Although all of the materials tested in these experiments provided excellent protection of logs from beetle invasion, there seems little doubt that the formulations most efficient in this respect were emulsions and solutions. Because of the results obtained, it is difficult to

Table 3.—The relative toxicity to Douglas-fir beetles of emulsions and suspensions of five insecticides applied to wood fiber surfaces.

Treatment (mg. active ingredient /sq. ft.)	Per Cent Mortality After (weeks)		
	0	4	7
Endrin, 75			
Suspension	80	62	31
Emulsion	42	20	0
Lindane, 75			
Suspension	100	15	0
Emulsion	61	8	0
Thiodan, 75			
Suspension	100	65	34
Emulsion	65	31	0
Sevin, 150			
Suspension	80	53	28
Emulsion	42	22	10
Heptachlor, 150			
Suspension	60	15	0
Emulsion	38	8	0

make a further choice between these two formulations. The superior residual properties of solutions compared with emulsions are evident in the case of heptachlor and lindane, where the amount of insecticide remaining during the 10 weeks was markedly greater with the solution. With endrin, however, the differences were less, with the emulsion showing slightly more residue than the solution. This reversal in residual effectiveness suggests that more work should be done in developing formulations for this type of insect control. It seems likely that two factors are important in the development of such formulations, the solubility of the pesticide in the solvents used, and the bark-penetrating characteristics of the solvent.

Practical use of any of these materials is justified by their performance under the conditions described. During the first 7 weeks after application of these materials, 3.5 inches of rainfall occurred, the rainy periods alternating with sunny periods in which temperatures were in the 80's. Beetle activity was high for the first 11 weeks and would in time have produced serious damage to the freshly cut logs, as indicated by the number of beetle invasions of the untreated sections.

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Field Tests with Insecticides for Control of Lone Star Ticks on Cattle[1]

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ABSTRACT

In 1958 and 1959 cattle were sprayed with 11 insecticides to control lone star ticks (*Amblyomma americanum* (L.)). The considerable variation in effectiveness of the standard, 0.5% toxaphene, at 1 week posttreatment was dependent upon reinfestation pressure. One week after treatment 0.5% Co-Ral® was most effective, 0.5% toxaphene, 0.75% ronnel, and 0.5% malathion afforded equal protection, and 0.025% lindane was the least effective of these recommended sprays. Of the newer compounds tested, 0.15% Delnav® and 0.5% Sevin® afforded greater reductions than toxaphene; 0.5% Bayer 22408 was as effective as the standard; and dicapthon, Dipterex® and Ruelene® gave least reductions. None of the treatments were effective after 2 weeks.

The chemical names of the compounds are Co-Ral (*O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate), Delnav (2,3-*p*-dioxanedithiol *S,S*-bis(*O,O*-diethyl phosphorodithioate) (68% to 70% and related materials)) Sevin (1-naphthyl methylcarbamate), Bayer 22408 (*O,O*-diethyl *O*-naphthalimido phosphorothioate), Dipterex (dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate), and Ruelene (4-*tert*-butyl-2-chlorophenyl methyl methylphosphoramidate).

Around Kerrville, Texas, compounds are evaluated against ticks by treating cattle infested with the lone star tick (*Amblyomma americanum* (L.)). Results of these tests and of others with different species of ticks in other parts of the United States are used to determine the insecticide recommendations of the Entomology Research Division for the control of ticks on cattle. Sprays of Co-Ral (*O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate), malathion, and ronnel, and sprays or dips of lindane or toxaphene are currently recommended (Entomology Research Division 1959).

In 1958 and 1959 tests were conducted on several ranches near Kerrville to compare the efficiency of sprays of the five recommended insecticides and six newer organophosphorus and carbamate insecticides for the control of lone star ticks on adult cattle. The new compounds are Bayer 22408 (*O,O*-diethyl *O*-naphthalimido phosphorothioate), Delnav® (2,3-*p*-dioxanedithiol *S,S*-bis(*O,O*-diethyl phosphorodithioate) 68% to 70% and related materials), dicapthon, Dipterex® (dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate), Ruelene® (4-*tert*-butyl-2-chlorophenyl methyl methylphosphoramidate), and Sevin® (1-naphthyl methylcarbamate).

METHODS.—Tests were conducted with small groups of cows. Individual animals were confined in chutes and the numbers of adult ticks on one side of the dewlap and of the escutcheon from the top of the tail head to the udder were recorded. The treatment groups were designated with cattle-marking dye. The animals were moved to small pens and sprayed with insecticides at the rate of 1 to 2 gallons per head at pressures of 100 to 250 pounds per square inch. One group at each ranch was treated with 0.5% toxaphene as a standard of comparison. Because of the large tick population none of the cows was left untreated.

After treatment all test animals were turned into the same pasture to insure equal exposure to ticks and were checked weekly. The effective control period of the insecticides was based on the length of time before ticks reached pretreatment levels. This was modified when reinfestation pressures decreased naturally.

TESTS IN 1958.—The most heavily infested cows at one ranch were placed in treatment groups of five and sprayed four times during the tick season. At another ranch cows were placed in three unequal groups and sprayed three times. The compounds used were Delnav from a 30% emulsifiable concentrate, Bayer 22408 and Sevin from 50% wettable powders, toxaphene from a 67% emulsifiable concentrate, and ronnel and Co-Ral from 25% wettable powders. The results of these tests are summarized in table 1.

TESTS IN 1959.—Cows from one herd were placed in

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Table 1.—Average number of adult ticks on cattle before and after spraying, 1958.

Spraying Date	Insecticide	Concentration (%)	Before Spraying	Weeks After Spraying			
				1	2	3	4
			Ranch I (5 cows checked each week)				
May 9	Bayer 22408	0.25	74.4	6.6	35.8	35.4	
	Delnav	.15	67.4	4.2	38.6	53.4	
	Sevin	.5	49.6	4.6	24.4	39.0	
	Toxaphene	.5	56.6	4.2	42.4	32.2	
May 29	Bayer 22408	0.25	34.5	25.4	53.5	49.0	
	Delnav	.15	53.4	21.8	40.2	34.2	
	Sevin	.5	39.0	19.4	45.6	31.6	
	Toxaphene	.5	32.2	15.4	47.6	28.4	
June 20	Bayer 22408	0.25	49.0	21.0	36.2	23.4	
	Co-Ral	.5	35.2	1.4	28.0	29.2	
	Delnav	.15	34.2	13.2	26.4	29.2	
	Sevin	.5	31.6	9.0	36.0	30.6	
	Toxaphene	.5	28.4	11.2	37.0	32.4	
July 11	Bayer 22408	0.25	23.4	4.0	13.4	4.8	2.8
	Co-Ral	.5	29.2	1.4	10.8	3.2	4.4
	Delnav	.15	29.2	1.4	9.0	6.2	4.2
	Sevin	.5	30.6	4.4	13.0	3.4	4.0
	Toxaphene	.5	32.4	3.2	13.4	2.0	3.6
			Ranch II (5–20 cows checked each week)				
May 6	Ronnel	0.75	3.7	0.5	5.0	4.1	
		.5	7.6	6.1	8.3	4.6	
	Toxaphene	.5	2.7	0.9	1.8	4.3	
May 27	Ronnel	0.75	4.1	6.3	1.1	10.2	4.7
		.5	4.6	9.5	4.1	1.8	4.1
	Toxaphene	.5	4.3	5.8	10.0	7.6	7.7
June 25	Ronnel	0.75	4.7	0.4	0.5	4.3	1.0
		.5	4.1	3.8	0.1	4.1	3.4
	Toxaphene	.5	7.7	2.1	5.1	5.6	0.4

Table 2.—Average number of adult ticks on cattle before and after spraying, 1959.

Spraying Date	Insecticide	Concentration (%)	Before Spraying	Weeks After Spraying			
				1	2	3	4
			Ranch I (5 cows checked each week)				
April 3	Bayer 22408	0.5	38.0	13.6	23.0	39.0	42.0
	Dicapthon	.5	36.2	28.0	25.6	44.6	44.8
	Malathion	.25	45.8	36.3	31.2	46.6	49.4
	Ronnel	.5	40.2	21.8	33.2	44.8	45.8
	Toxaphene	.5	46.0	4.4	14.6	30.4	24.4
April 30	Co-Ral	0.25	40.6	19.6	63.4	55.4	
	Dipterex	.5	42.4	24.0	61.4	35.8	
	Malathion	.5	37.4	17.0	37.4	46.4	
	Ronnel	1.0	48.2	31.8	71.4	61.2	
	Toxaphene	.5	39.4	19.0	90.6	61.6	
May 22	Co-Ral	0.5	46.4	12.6	38.0	24.0	
	Delnav	.15	35.8	30.4	56.8	49.0	
	Lindane	.025	55.4	44.2	66.0	32.6	
	Ronnel	.75	61.2	36.0	56.4	29.4	
	Toxaphene	.5	61.6	17.2	55.4	32.4	
June 12	Ronnel	0.75	30.0	12.2	16.8	11.2	8.2
	Ruelene	.25	45.4	19.8	15.2	12.8	9.8
		.5	32.0	16.8	17.4	16.6	11.0
	Toxaphene	.5	23.6	4.8	9.6	11.2	10.5
			Ranch II (10 cows checked each week)				
May 19	Ruelene	0.5	17.3	11.2	21.6	18.2	
	Toxaphene	.5	18.7	3.1	13.6	12.2	

groups of five and sprayed four times. The animals were regrouped before the second and fourth sprayings. Cows from a second herd were placed in groups of 10 and sprayed once. The compounds used were dicapthon from a 25% emulsifiable concentrate; malathion from a 57% emulsifiable concentrate; Dipterex from a 50% soluble powder; Ruelene and lindane from 25% wettable powders; and Bayer 22408, Co-Ral, Delnav, ronnel, and toxaphene from the same formulations used in 1958. The results are summarized in table 2.

DISCUSSION.—Considerable variation was evident in the residual effectiveness of the standard treatment, 0.5% toxaphene. This variation can be explained on the basis of the seasonal biology of the lone star tick. In the Kerrville area ticks usually appear on cattle in February and reach peak numbers in late May or early June. Numbers of ticks remain high during June, but with the advent of hot, dry weather there is a decrease during the remainder of the summer. When cattle were sprayed with toxaphene early or late in the tick season, populations were reduced 70% to 90% after 1 week, but treatment during periods of heaviest reinfestation pressure reduced populations not more than 63%.

Since not all the insecticides were used on each treatment date, they were exposed to different levels of reinfestation. To allow for this variation, the data in table 3 are grouped in terms of average per cent reduction of pretreatment populations. The table does not include the treatments applied late in the season, after which populations did not approach pretreatment levels. In most tests the reduction was not great after the first week.

Of the recommended treatments after 1 week 0.5% Co-Ral afforded the most reduction. 0.5% malathion and 0.75% ronnel were slightly less effective than toxaphene, and lindane afforded the least reduction. After 2 weeks Co-Ral, ronnel, and toxaphene were equally effective, and lindane and malathion were ineffective.

Of the new compounds Delnav and Sevin gave the greatest protection after 1 week, both concentrations of Bayer 22408 were equal to toxaphene; and dicapthon, Dipterex, and Ruelene were the least effective. After 2 weeks 0.5% Bayer 22408 was the most effective; Delnav, Sevin, and dicapthon were as effective as toxaphene; and Ruelene and Dipterex were ineffective.

Table 3.—Average (range) per cent reduction in populations of lone star ticks on cattle after spraying.

INSECTICIDE	CONCENTRATION (%)	AFTER SPRAYING (WEEKS) 1	2	3
Bayer 22408	0.25	62 (29–91)	26 (0–52)	52
	.5	63	40	0
Co-Ral	25	52	0	
	.5	85 (73–96)	19 (18–20)	17
Delnav	.15	75 (15–94)	25 (0–43)	26 (15–36)
Dicapthon	.5	23	29	0
Dipterex	.5	43	0	
Lindane	.025	20	0	
Malathion	.25	20	0	
	.5	55	0	
Ronnel	.5	15 (0–46)	8 (0–17)	0
	.75	55 (0–91)	32 (0–89)	9
	1.0	34	0	
Ruelene	.5	35	0	
Sevin	.5	71 (50–91)	17 (0–51)	0
Toxaphene	.5	64 (0–92)	22 (0–68)	26 (0–43)

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New Approved Common Names of Insecticides. II.

CARROLL N. SMITH, *Chairman, Committee on Insecticide Terminology*

A revised list of common names of insecticides approved for use in the publications of the Entomological Society of America was published in the April 1959 issue of the JOURNAL OF ECONOMIC ENTOMOLOGY (**52**: 361–2). Changes and additions to the list were published in the October 1959 and August 1960 issues (52: 1032 and **53**: 677). The additional new name listed below has been approved by the Committee on Insecticide Terminology of the Entomological Society of America:

NAME TO BE USED	CHEMICAL DEFINITION
butonate	*O,O*-dimethyl 2,2,2-trichloro-1-*n*-butyryloxyethyl phosphonate

SCIENTIFIC NOTES

The Effectiveness of Liquefied-Gas-Propelled Concentrated Allethrin Aerosols and Air-Atomized Dibrom Aerosols Against Normal and Resistant House Flies[1]

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Recent experiments at Beltsville, Maryland (Schechter *et al.* 1960) demonstrated that 1% and 10% allethrin aerosols gave approximately the same mortality of house flies (*Musca domestica* L.) when equal dosages of allethrin were used. This is an important finding in military and other uses where logistics is a prime consideration, because the 10% aerosol has a high killing power per unit of weight and volume.

An objective of the present work was to determine whether the aerosol formulation could be further concentrated without a loss in effectiveness and still produce a satisfactory aerosol. A further objective was to find an insecticide that might be useful in coping with extremely resistant flies.

Dibrom (1,2-dibromo-2,2-dichloroethyl dimethyl phosphate) was selected as a test insecticide because it had been reported to have low acute mammalian toxicity and had shown promise in screening tests. Corrosion difficulties with liquefied gas-propelled aerosol formulations made it necessary to test this material as an atomized aerosol. This was done by placing 2% (weight/volume) Dibrom in a glass container and atomizing in the aerosol particle-size range (approximately 17.5 microns m.m.d.) with a Peet-Grady type DeVilbiss atomizer at an air pressure of 100 p.s.i.

Allethrin was selected because it is relatively harmless to warm-blooded animals, it is effective against flies and mosquitoes, and there are no formulation problems at high concentrations.

Ten per cent, 15% and 20% allethrin formulations were made up as follows:

	I	II	III
Diameter of particles (microns):			
Mass median	17.5	21.5–25.5	20–24.5
Largest	38	42–69	42–70
Per cent of allethrin (93.3%)	11.1	16.7	22.2
Per cent of propellent:			
11	44.45		
12	44.45	83.3	77.8

In formulation I, a 50:50 mixture of propellents 11 and 12 was used and all of the particles were in the aerosol range. In II and III, propellent 12 was used alone to give a pressure high enough to atomize the allethrin into particles that were mostly in the aerosol range. Actually, 80% or more of the particles were smaller than 30 microns, but there was a range of 0% to 3% of particles over 50 microns with individual bombs.

All tests were made in 1000-cubic foot chambers at temperatures from 20° to 26° C. Four strains of flies were used in the tests. The National Association of Insecticide and Disinfectant Manufacturers (NAIDM) 1948 normal and Orlando-Beltsville resistant strains of house flies are in common use. The Fales' 1958 wild flies are a mixed strain from insects collected from all parts of the U.S.A. The Beltsville resistant 1959 strain was collected at Beltsville in 1959. After the flies had been acclimated in the chambers for 15 minutes, they were exposed for 30 minutes to the test aerosol. Both free-flying and knocked-down insects were then placed in recovery cages for a 24-hour observation period. All but two of the tests (Dibrom—13 and 27 mg.) were repeated from two to four times.

The results of these tests are shown in table 1. From them the following conclusions are drawn:

Table 1.—Effectiveness of liquefied gas-propelled concentrated allethrin aerosols and atomized Dibrom aerosols against several strains of house flies.[a]

Strain of Flies	Active Ingredient (%)	Active Ingredient (Mg./1,000 Cu. Ft.)	No of Flies	Knockdown (%) in 3 Min.	Knockdown (%) in 30 Min.	Mortality in 24 Hrs. (%)
Allethrin Aerosols						
Normal NAIDM 1948	10	52	923	30	96	96
		152	776	35	91	97
	20	50	848	28	91	88
		163	249	40	98	99
Fales' 1958 Wild Flies	10	51	497	27	90	65
		164	407	38	95	85
		307	862	45	96	97
	20	55	529	32	92	81
		151	318	53	97	97
		304	985	56	97	97
Orlando-Beltsville Resistant	10	304	500	1	76	62
	15	305	473	1	74	64
	20	303	569	1	78	65
		212	466	1	53	28
		338	263	11	84	77
		561	401	22	78	58
		936	580	37	95	94
Dibrom Aerosols						
Normal NAIDM 1948	2	13.1	400	2	94	94
		26.4	585	3	100	100
		50.5	350	7	100	100
Orlando-Beltsville Resistant	2	14	268	1	80	83
		28.4	204	1	96	96
		53.5	1,076	4	99	99
Beltsville Resistant 1959	2	13.0	145	0	87	86
		27.0	174	1	99	99
		50.7	464	3	100	99

[a] Flies exposed for 30 minutes in a 1000-cu. ft. chamber at 20° to 26° C.

1. Concentrated allethrin aerosols show promise in situations that demand maximum performance per unit weight.

2. There appears to be no difference in effectiveness between the 10%, 15% and 20% allethrin aerosols when equivalent amounts of active insecticide are used.

3. Allethrin and Dibrom are very effective against normal flies but Dibrom is superior against resistant flies.

4. Dibrom looks particularly promising for military and agricultural uses in the field provided toxicological clearance is obtained.

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Schechter, Milton S., W. N. Sullivan, and Alfred H. Yeomans. 1960. Studies on fine-particle, coarse-particle and concentrated aerosols. Jour. Econ. Ent. **53**(5): 908–14.

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[2] Grateful acknowledgment is made to R. A. Fulton and J. C. Keller for helpful suggestions, and to T. R. McCauley for carrying out many of the tests.

Seed Abortion and Fruit Drop in Bartlett Pear Caused by Sevin[1]

Everett C. Burts and Samuel G. Kelly[2]

During the 1959 season, Sevin® (1-naphthyl methylcarbamate) and three other insecticides were applied in replicated, randomized plots to a block of 15-year-old Bartlett pear trees. Sprays containing 2 pounds of 50% Sevin wettable powder per 100 gallons of water were applied to plots on May 11, June 2, and July 17. The first spray was applied 21 days after the trees

[1] Accepted for publication March 11, 1960.
[2] Assistant Entomologist and Senior Experimental Aide, respectively. Washington State University, Tree Fruit Experiment Station, Wenatchee, Washington. Work was conducted under Project No. 1187.

were in full bloom. Just before harvest, a much heavier drop of fruit was observed under trees in plots sprayed with Sevin than under trees in the other plots. There was an average of 237 fruits per tree on the ground in the Sevin plots compared with only 63 in the bordering plots. The drop of fruit in the Sevin plots might have been even heavier except that the crop was harvested early to prevent additional loss. The fruit on trees in the Sevin plots was very loose at harvest time and much of it would fall when touched.

In Central Washington, some of the blossoms of Bartlett pear will develop fruits with no, or only a few seeds, especially where conditions for pollination and fertilization are poor. These seedless fruits lack the typical Bartlett shape, being much thicker necked. They also tend to drop from the tree just prior to harvest. In order to determine if the excess drop of fruit in the Sevin plots was associated with a reduction of seeds, counts of seeds were made in 250 fallen and 250 picked fruits from the Sevin plots and from the bordering plots. The results of the seed count are as follows:

Treatment	Type of Fruit	Seedless Fruit (%)	Average Seeds per	
			Fruit	Seeded Fruit
Sevin	Dropped	30.9	1.1	1.5
	Picked	30.1	1.4	2.0
Untreated	Dropped	33.3	1.2	1.9
	Picked	18.8	2.2	2.7

As shown in the table, Sevin caused a reduction in the number of seeds in the fruit and an increase in the percentage of seedless fruit. The lack of seeds in turn caused much of the fruit to drop just prior to harvest. However, fruit from trees in the other plots had a low seed count and there was considerable fruit drop throughout the orchard. This low initial seed count is probably why the effect of Sevin was expressed so clearly in this orchard.

Batjer & Westwood (1960) found that sprays containing Sevin applied to Delicious apple trees 16 to 19 days after full bloom were effective in thinning the fruit. They also found that apples remaining on the trees after this treatment had fewer seed than fruit from unsprayed trees. It appears that the effect of early post-bloom sprays of Sevin on these two pome fruit varieties is similar except that the seed abortion in apples causes an early drop, whereas in pears it results in fruit drop close to harvest time.

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A Fungus Disease of the Salt-Marsh Caterpillar In Arizona[1]

George P. Wene, *University of Arizona*, and L. W. Sheets, *Entomology Research Division, Agric. Res. Serv., U.S.D.A.*[2]

The salt-marsh caterpillar (*Estigmene acrea* (Drury)) was, as usual, abundant in the Salt River Valley of Arizona in September 1958, although a few fourth- or fifth- instar larvae dead from disease were seen clinging to heavily infested branches of cotton. A more severe caterpillar infestation developed in October, but the potential population was materially reduced by this disease, the causative organism of which was determined as *Entomopthora aulicae* (Reich) by C. G. Thompson of the U. S. Department of Agriculture Insect Pathology Laboratory.

This disease was found in irrigated cotton shortly after the last September irrigations. During the last half of September and the month of October the weather was considered as "humid" for this area.

According to Thompson (1959) this disease, in nature, affects salt-marsh caterpillar populations from coast to coast, usually in areas of considerably higher prevailing atmospheric moisture than Arizona. Steinhaus (1949) stated that the disease attacks the caterpillars of the brown-tail moth (*Nygmia phaeorrhea* (Donov.)), as well as the larvae of more than a dozen other species, but he did not specifically mention the salt-marsh caterpillar.

Field observations by the writers indicated no visible evidence that caterpillars were affected by the disease before they reached the third-instar, although normal feeding activity of older larvae was greatly decreased. An examination in one field, in October 1958, indicated that 70% of the larvae beyond the second-instar had been killed by the disease. In other fields, practically all larger salt-marsh caterpillars were dead, dry and brittle. The disease was so effective that but few fields required insecticidal control of the caterpillars for the remainder of the season, and the usual late-season migration from cotton to vegetable fields was absent.

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[1] Journal Paper. University of Arizona Agricultural Experiment Station. Accepted for publication April 28, 1960.

[2] The writers are grateful to C. G. Thompson for the diagnosis of the disease and for other information, and to F. F. Bibby for invaluable collaboration.

Control of Maple Leaf Cutter, *Paraclemensia acerifoliella*, by Aerial Spraying[1]

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The maple leaf cutter, *Paraclemensia acerifoliella* (Fitch), has caused serious damage to the foliage of sugar maple, *Acer saccharum* Marsh., in northern New York during 1958 and 1959 especially in the Lowville area where considerable damage occurred in sugar orchards.

According to Craighead (1950), the moths emerge in May and deposit their eggs in the tissues of the leaves. After egg hatch, the young larvae feed as leaf miners for 10 days or 2 weeks and then cut oval-shaped cases out of the mines and live as case-bearers, feeding on the epidermal layers of leaf tissue. Whenever necessary to enlarge their cases, the larvae cut larger oval pieces out of the leaves and add them to their cases. The larvae become full grown in late August or September and fall to the ground to pupate in their cases. There is one generation per year. Damage by the insect causes the foliage to lighten in color and later to turn brown. Such damage causes a reduction in tree vigor and a reduction in the sugar content of the sap.

On July 22 and 23, 1959, six sugar orchards of 300, 125, 100, 70, 60 and 50 acres in size were sprayed with 9% by weight of DDT in No. 2 fuel oil at the rate of 2 gallons per acre.[2] The spray was timed to be applied after the larvae had ceased mining and were feeding on the epidermal layers of the leaves and before serious damage to the foliage occurred.

The aircraft equipment used was a 1958, PA 18-A, 150 Super Cub high wing, single engine monoplane equipped with a 110-gallon spray tank and an externally mounted 34-foot spray

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[2] Dosage was suggested by E. B. Walker, Vermont Forest Service, from tests made in that state.

boom. The boom was equipped with 24 Spraying Systems diaphragm *T-jet* nozzles with D4 orifices and No. 45 cone-shaped cores, 12 under each wing. The boom was mounted 3 feet below the trailing edge. Normal operating (boom) pressure during spraying was 40 p.s.i. The plane was calibrated to deliver 2 gallons of spray per acre (about 14 gallons per minute) when flying at 70 m.p.h. at 25 feet above the tree tops. This gave a 50-foot swath over the forest canopy.

Weather conditions, at the time of spraying, were satisfactory for aircraft spraying. The air was cool and stable with occasional movements of less than 2 m.p.h.

The DDT spray solution was prepared by dissolving technical DDT directly in the fuel oil. Since air temperatures on the date of application remained above 40° F. there was no danger of the DDT precipitating out of solution.

The results of the spraying were evaluated on August 15 by an aerial inspection. The foliage of sprayed sugar orchards was green compared with the browning foliage of adjacent unsprayed sugar maple stands. Ground inspections revealed that in sprayed sugar orchards the leaf cutter activity had not progressed any further than it had on the day of spray application, whereas activity in unsprayed stands had continued until the foliage was badly damaged and turning brown.

Cost of spray application was $2.60 per acre. The spraying was done by Thomas E. Duflo of New Bremen, N. Y.

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The Nesting Habits of the Wasp, *Solierella* sp., and the Location of Its Egg on the Grasshopper, *Ageneotettix deorum*[1]

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A medium-sized wasp belonging to the family Sphecidae, genus *Solierella* sp.,[2] was observed to provision its burrow with a fourth-instar nymph of *Ageneotettix deorum* (Scudder) on rangeland in Wheatland County, Montana, on July 6, 1956. As this was the only *Solierella* observed during the 1954 to 1956 seasons, and because the wasp attached its egg at a different location on its prey than do *Tachysphex* digger wasps, the few habits noticed are reported.

Muesebeck *et al.* (1951) list 36 species of *Solierella* and say there is considerable variation in the nesting sites and prey used within the genus. According to them, some species provision burrows with nymphs of the Oediopodinae (Orthoptera), whereas others store nymphs of Lygaeidae (Hemiptera) in nests made in twigs and deserted galls. Williams (1950) observed a number preying on grasshoppers. He noted *S. striatipes* (Ashmead) using an adult brachypterous female *Melanoplus ligneolus*, and on another occasion the species used one adult and one immature melanopine grasshopper. He reported *S. similis* (Bridwell) and possibly *S. vandykei* Williams as using immature grasshoppers and stated that *S. fossor* (Rohwer) had been collected with an oedipode grasshopper.

The *Solierella* sp. in Montana was first noticed as it entered a ¼-inch-diameter hole that extended vertically into the soil. The entrance lacked the pile of excavated soil that is characteristic of *Tachysphex* digger wasps. Williams (1950) stated that the *Solierella* show little construction ability and use the burrows of other insects. The *S. striatipes* that he studied used an abandoned trap-door spider or tarantula burrow, and *S. similis* used a broken hollow stem of an umbelliferous plant. It is possible that the wasp observed by the writer was also using a borrowed hole. In less than 1 minute it reappeared and walked directly to a clump of grass about a yard away and immediately returned dragging a paralyzed fourth-instar *A. deorum*. It is not known whether the wasp captured its prey on this trip or had taken it previously and placed it nearby. On reaching the burrow opening, the wasp entered with the grasshopper. Within 2 minutes it reappeared and began scouting a square-yard area for loose fragments of dried stems, grasshopper frass, and small pebbles which it carried one at a time in its mandibles and packed into the hole—a task that took about 15 minutes. When the closure appeared to be complete, the writer captured the wasp for identification and then reopened the burrow. It was found to be about 1 inch deep, slightly enlarged at the end, and contained but the one nymph which then had a 2-mm.-long pale gray egg attached back of the left metathoracic coxa and extending ventrally (fig. 1). Although the nymph with attached egg was placed in a small can with moist soil, the egg shriveled and died on the third day whereas the grasshopper remained alive an additional day.

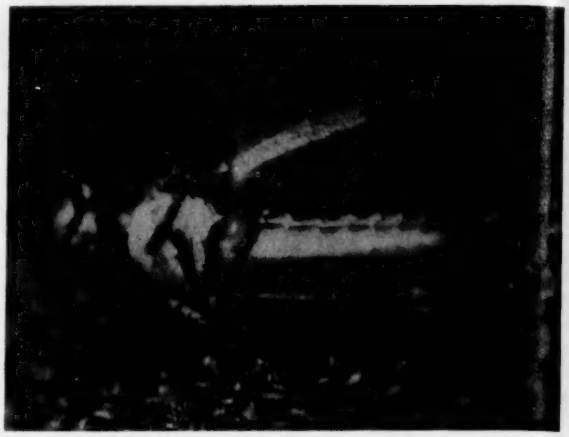

FIG. 1.—Paralyzed fourth-instar nymph of *Ageneotettix deorum* showing the egg of *Solierella* sp. placed back of the left coxa. About 3×.

The location of this *Solierella* egg on *A. deorum* appears to be consistent with observations by Williams (1950). He found that in *S. striatipes*, which had cached one adult and a nymph together, "The pale creamy *Solierella* egg was firmly cemented at one end, in the crotch formed by the inner base of the hind legs and the body, on the right side in one victim, and on the left in the other. It protruded upwards to bend somewhat over the venter of the first abdominal segment of the grasshopper." In *S. similis* Williams found "a single nymph with a *Solierella* egg glued to the base of the abdomen and just back of the stub of the hind leg and therefore in about the same situation as that of *S. striatipes*." Again in *S. similis* or *vandykei*, which resembles it, he noted that one of the three immature grasshoppers " . . . bore the delicate wasp egg glued near the base of one hind leg and extending obliquely across the breast." This position is in close agreement with that of the egg pictured on *A. deorum* (fig. 1).

Since the position of eggs of *Solierella* wasps appear to be consistent on grasshopper prey, it can serve as an identification in the absence of the adult predator. This is likewise true for the digger wasps of the genus *Tachysphex* and *Prionony*x, both of which were collected on Montana rangeland with *Solierella*. In *Tachysphex* the egg is consistently placed ventrally between the prolegs of its prey (Newton 1956) and, according to Evans (1958), in the digger wasp *Prionony*x the egg is usually placed just above the coxa and curves downward over the base of the femur.

[1] Accepted for publication May 2, 1960.

[2] Determination made by K. V. Krombein, Entomology Research Division.

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A Ventilation Unit for Aerating Insectary Sleeve Cages[1]

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When culturing plant feeding insects in the insectary it becomes necessary at times to hold potted plants under caged conditions for a considerable length of time. Occasionally it would be desirable to use conventional sleeve cages to enclose the plants infested with host pest insects together with the parasites or predators. Providing an environment favorable to both the plants and insects presents a real problem especially when honeydew producing host insects such as leafhoppers or aphids are involved. Unless the cage is well ventilated the air inside becomes humid and stagnant, the plant leaves tend to turn yellow and drop, and sooty mold fungus develops on the honeydew excreted by the insects (Kennedy & Booth 1950). Furthermore the ovipositional activity of some species of parasites is sharply reduced under humid conditions rendering them much less effective.

In 1954 the University of California's Department of Biological Control at Albany became engaged in extensive mass rearing of egg parasites of the beet leafhopper, *Circulifer tenellus* (Baker). In the course of this project a unit designed to ventilate the insectary sleeve cages was built and used with very beneficial results (fig. 1). In 1956 the unit was modified and has since been used extensively in the culture of certain parasites of the spotted alfalfa aphid, *Therioaphis maculata* (Buckton) and the pea aphid *Macrosiphum pisi* (Harris).

The ventilation system is constructed of $\frac{3}{4}$-inch fir plywood and consists of a tunnel 6′ and 2″ long with inside measurement 10 inches square. The top and bottom boards of the tunnel are $17\frac{1}{2}$ inches wide thus extending beyond the side boards 3 inches on either side. The tunnel is closed by a $10\times17\frac{1}{2}$ inch board at one end and open at the other. A fan is installed in a housing $17\frac{1}{2}$ inches square and 6 inches deep built on the tunnel so as to pull the air from its open end. Six strips of $\frac{3}{4}$-inch plywood $2\frac{1}{4}$ inches wide and 10 inches long are installed, three on each side of the tunnel and at right angles to it partitioning each side into four equal sections. Strips of $\frac{3}{4}$-inch plywood $2\frac{1}{2}$ inches wide and

[1] Accepted for publication February 17, 1960.

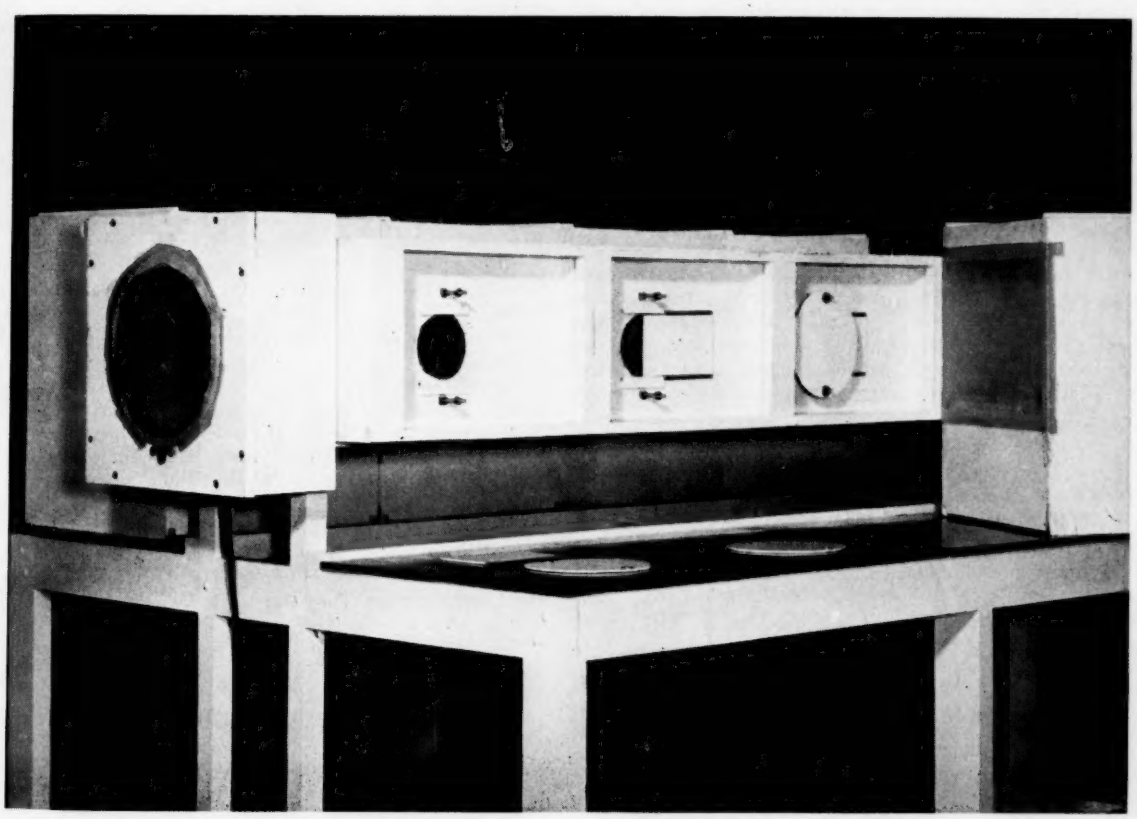

FIG. 1.—Ventilation line showing fan housing and three of the four sections which extend from each side of the tunnel. The fourth section is hidden by the sleeve cage which is shown pulled away from the third section which is fully assembled. The second section is shown with the baffle disc removed exposing the slide arrangement over the port. The first section has both disc and slide removed. Four cages may be observed backed against the opposite side of the tunnel.

10 inches long are then installed at right angles to each of these dividing strips, parallel to them, fitting between and flush with the edges of the extended top and bottom tunnel boards. In addition four more strips 10 inches long and 1½ inches wide are likewise installed flush with the top and bottom boards, two on either side of the tunnel next to the fan box and the other two on either side at the other end of the tunnel. Thus four sections 3 inches deep are formed on either side of the tunnel against which eight sleeve cages may be set so that the 10- by 18-inch cloth or screen covered opening in the back of each will fit against and match each section.

In the center of the back of each section a hole or "port" 4 inches in diameter is cut through the tunnel wall. A piece of ⅛-inch pressboard 4½ inches by 7 inches is installed over each port in slotted guides so that it will slide back and forth covering all or any portion of the aperture thus regulating the flow of air drawn into the tunnel. A disc 8 inches in diameter made of ⅛-inch pressboard is installed over each aperture and held in a position about 2 inches away from the side of the tunnel by means of locknuts on two threaded bolts protruding 2½ inches from the side of the tunnel and about 5½ inches apart one above and one below the regulating slide. The discs serve to distribute more evenly the flow of air drawn through the cloth or screen backs of the cages.

To put the tunnel unit into an operational position it is laid lengthwise on the middle of a table at least 6 feet long and 56 inches wide. The tunnel is lifted and fixed at an elevation that will permit the 10- by 18-inch openings in the back of the sleeve cages to match and seal against each section constructed on the sides of the tunnel when the cages are backed up against them. A more positive seal could be obtained by gluing a gasket of foam rubber strips, one-half inch thick, around each tunnel section.

Holes stoppered with corks or slots regulated by slides, could be cut in the lower part of the cage doors to further regulate the amount of air entering the cages, however the volume drawn through the cloth sleeves has usually been adequate. The volume of air drawn through a cage may be adjusted by means of the port regulator slide so that only the amount of air will flow that is adequate to maintain the same humidity in the cage as that maintained in the room. The humidity controls for the room may thus serve to regulate the humidity within the cage as well.

This unit may also be used to raise the humidity in sleeve cages above that of the room by circulating cool, humid air through them. To accomplish this the fan is either removed from the tunnel or left inoperative. A small evaporative cooler, preferably one with a rotor type fan, is mounted so that it blows the air into the tunnel. Thus cool humid air is forced into each cage set against the unit. The air entering the cages may be regulated by adjusting the air flow control slides across the ports on the sides of the tunnel.

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Persistence of BHC, Lindane, and Thiodan Residues When Applied to Alfalfa to Control the Meadow Spittlebug[1]

JACK E. FAHEY,[2] M. CURTIS WILSON,[3] and HAROLD W. RUSK[2]

BHC, lindane, and Thiodan® (6,7,8,9,10,10-hexachloro-1,5,-5*a*, 6, 9, 9*a*-hexahydro-6, 9-methano-2, 4, 3-benzodioxathiepin-3-oxide) have been shown to be effective controls for the meadow spittlebug (*Philaenus leucophthalmus* (L.)) on forage legumes when applied at the rate of one-quarter pound per acre. The presence of residues on forage intended as feed for dairy cattle, or for cattle being finished for market, could result in contamination of milk or meat. Studies were conducted in 1958 and 1959 in Indiana to determine under what conditions these insecticides could be employed for spittlebug control and avoid contamination of alfalfa.

Table 1.—Parts per million of BHC at different intervals after application of BHC spray or granules or lindane sprays, 1959.[a]

INSECTICIDE APPLIED APRIL 29	ACTIVE INGREDIENT PER ACRE (LB.)	DATE SAMPLED	RESIDUE (P.P.M.)
BHC spray	0.22	April 30	6.1
		May 3	1.7
		8	1.3
		16	1.5
		24	0.3
		31	0.4
BHC granules	.26	April 30	1.4
		May 3	1.4
		8	0.6
		16	0.5
		24	0.6
		31	0.3
Lindane spray	.22	April 30	3.8
		May 3	1.4
		8	0.9
		16	0.6
		24	0.7
		31	0.1

[a] Average of four analyses.

METHODS.—Plots were laid out in alfalfa fields for the study of residues from insecticide sprays and granule applications. The sprays were applied with a compressed air (carbon dioxide) sprayer mounted on bicycle wheels and equipped with a 10-foot boom for forage spraying. The sprayer, pushed over the plots at a rate of 2 miles per hour and at a pressure of 40 p.s.i., delivered 10 gallons per acre. Granulated materials were distributed with a grass-seed box mounted on bicycle wheels. In 1958 plots 20×100 feet and placed in consecutive strips were treated with BHC and lindane formulations. One application was made on April 29, at which time the alfalfa was 12 to 15 inches in height. In 1959 the plots measured 20×80 feet and were separated by untreated border strips 20 feet wide. Sprays, prepared from Thiodan, lindane, and BHC emulsions, were applied at three stages of growth, 3, 6, and 12 inches in height.

Four 100-stem samples, representing 400 to 800 grams of mature alfalfa, were taken from each plot for residue analysis. Each sample was collected at random from the entire treated area. The unit of 100 stems varied in weight from 146 (3 in. alfalfa in 1959) to 960 grams (43 in. alfalfa in 1958).

To recover the residue from the treated samples, the alfalfa was cut into short lengths and placed in a 1-gallon jar with an equal weight of anhydrous sodium sulfate and 2 ml. of appropriate solvent per gram of alfalfa. The jar was then sealed and tumbled for 2 hours. The solution was then decanted into bottles and stored in a refrigerator until analyzed.

Residues of BHC and lindane were recovered from alfalfa samples by extraction with purified Skellysolve B (acid-washed and distilled). In the analysis of small aliquots equivalent to 20 grams of alfalfa or less, no preliminary "clean-up" was required. With larger aliquots the solvent was evaporated and the residue dissolved in a 6:1 mixture of methylene chloride and acetone, treated with 0.5 gram activated charcoal, filtered, and the solvent

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Table 2.—Residues on alfalfa sprayed with BHC, lindane, or Thiodan on different dates, 1959.[a]

Insecticide	Date Applied	Active Ingredient per Acre (lb.)	Date Sampled	Residue (p.p.m.)
BHC	April 6	0.18	April 6	17.7
			16	4.4
			May 19	0
	16	.19	April 16	14.8
			29	2.2
			May 19	0
	29	.23	April 29	14.0
			May 9	1.7
			19	0.4
Lindane	April 6	.21	April 6	7.0
			16	1.3
			May 19	0
	16	.28	April 16	7.9
			29	1.2
			May 19	0
	29	.30	April 29	8.3
			May 9	2.3
			19	0
Thiodan	April 6	.20	April 6	6.1
			16	1.0
			May 19	0
	16	.25	April 16	2.2
			29	0.1
			May 19	0
	29	.26	April 29	2.2
			May 9	0.2
			19	0.4

[a] Height of alfalfa: 3 inches on April 6, 6 inches on April 16, and 12 inches on April 29, (Average of 4 analyses).

evaporated. Final analyses were made by the Schechter-Hornstein method (1952), which depends upon the dechlorination of the BHC, nitration of the resulting benzene to *m*-dinitrobenzene, and the formation of a violet-red color with methyl ethyl ketone in the presence of strong alkali.

Thiodan residues were recovered in purified Skellysolve B and analyzed for organic chlorine by the sodium reduction technic of Phillips & De Benedictis (1954), followed by amperometric titration. Residues were calculated from chlorine analysis by applying the factor 1.91.

RESULTS.—The results of the 1958 studies (table 1) show that if BHC or lindane is applied to alfalfa 15 inches high, appreciable residues will remain after 24 to 31 days.

The results of the analysis of samples from the 1959 experimental plots (table 2) show that lindane sprays, applied to alfalfa not over 12 inches in height, resulted in no measurable BHC residues at maturity of the alfalfa 20 days later. BHC or Thiodan, applied when the alfalfa was 12 inches high, resulted in residues of 0.4 p.p.m. on mature alfalfa. When sprays were applied to alfalfa 6 inches high, there were no measurable residues on the mature alfalfa 33 days later.

The analytical method employed in BHC analysis does not differentiate between the several isomers of BHC found in commercial formulations. Technical lindane is approximately 99% gamma BHC; technical BHC, on the other hand, may contain 50% or more of other isomers. Thus, while the gamma isomer content of BHC and lindane sprays applied were approximately equal, the total BHC content of the BHC spray was considerably greater than that of the lindane spray. This difference accounts for the fact that the residue from BHC was about twice as great as that from lindane.

For the most practical control of the meadow spittlebug treatments should be applied at least 30 days before harvest. The data indicate that Thiodan or BHC applied at 0.2 pound per acre will not contaminate the alfalfa at harvest. If treatment must be delayed, lindane can be employed up to 20 days before harvest, when harvested at the 10% bloom stage.

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Biology of the Periodical Cicada in Georgia[1]

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Because of the long interval between population emergences, opportunities for observations on fecundity and adult biology of the 13-year periodical cicada, *Magicicada septendecim* (L.) race *tredecim* Riley, present themselves only rarely. In view of this fact, it is felt that the following observations will be of some value.

Brood XIX of the 13-year periodical cicada emerged in Georgia in 1959. Emergence began in mid-April and continued into early May. Reports of emergences of this insect were received from 14 counties in the State, the southernmost record being from Coffee County, about 75 miles north of the Florida line. At the peak of emergence the lower branches of the trees were laden with cast nymphal skins, and the adults could be seen at all levels in the trees. The appearance and sound of this cicada at peak emergence have been recorded by others (see Williams 1958).

In the emergence area studied, the lower branches of the trees and bushes were heavily marked by oviposition scars. On May 7 several small limbs were cut off and brought into the laboratory for the purpose of making egg counts. Ten different species of trees, all from a small area of a creek bottom woods in Oglethorpe County, Georgia, were represented in the limbs collected and are listed in table 1.

The mean number of eggs per clutch for all 10 host species combined is 18.36. This is above that found by Beamer (1931) who reported a mean of 15+ eggs per clutch for the 17-year periodical cicada in Kansas. On the other hand, Asquith (1954) found an average of 22 eggs per slit in counts made from a single young apple tree in Pennsylvania.

Marlatt (1907) indicated that some 80 plant species had been reported as oviposition hosts for the 17-year cicada. A number of trees have been reported by various authors as preferred oviposition hosts. Among those listed in the literature are apple, pear, and peach (Asquith 1954); beech, green ash, white oak, and elms (Jacobs 1954); and oaks, hickories, and apple (Marlatt 1907). However, no actual counts were made in any of these studies as to the number of eggs deposited in the different hosts. Although no data were included, Hardy (1947) noted evidence of more eggs being deposited in apple, hawthorne, oak, and hickory when these trees were intermixed with others.

A statistical analysis to determine if significantly more eggs were deposited per clutch in one host species than another was carried out (table 2) for the 10 host species studied. Except for box elder maple, the counts made here showed no generally consistent difference in the number of eggs per clutch among the species of host plants. The results of the analysis show that, compared with six of the nine other plants, significantly more eggs were deposited per clutch in box elder maple. From general

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Table 1.—The number of eggs laid by the periodical cicada in different species of trees.

Host Plant No.	Host Plant Common Name	Host Plant Scientific Name	No. of Egg Clutches Counted	Mean No. of Eggs per Clutch	Standard Error of Mean
1	Blue beech	*Carpinus caroliniana* Walt.	20	16.8	1.81
2	Willow oak	*Quercus phellos* L.	19	18.3	1.82
3	Box elder maple	*Acer negundo* L.	42	21.3	0.72
4	Flowering dogwood	*Cornus florida* L.	15	19.5	2.13
5	Redbud	*Cercis canadensis* L.	21	15.5	1.38
6	Winged elm	*Ulmus alata* Michx.	31	18.3	1.31
7	Sweet gum	*Liquidambar styraciflua* L.	39	17.9	1.21
8	Ash	*Fraxinus sp.*	19	15.8	1.97
9	Deciduous holly	*Ilex decidus* Walt.	7	21.1	3.16
10	Swamp dogwood	*Cornus sp.*	48	18.2	1.21

observation, also, it appeared that more clutches were deposited per lineal foot in box elder maple. Although the largest mean number of eggs per clutch was in deciduous holly, the large standard error, probably due to the small sample, undoubtedly accounts for the lack of significant differences in the number of eggs per clutch for this host as compared with other hosts.

The periodical cicada female deposits her eggs in a V-shaped groove cut in the plant tissue. Counts of the number of eggs deposited in each arm of the V showed some variations, but no significant difference was found.

It should be pointed out that the oviposition and fecundity data are based on a large and a small form considered as a single population. These forms may eventually be classed as distinct species. No study was made to determine if the eggs of the two forms could be distinguished, although Beamer (1931) stated that the eggs of *cassinii* were slightly the smaller in the 17-year race.

Small twigs containing oviposition slits were set in tap water and held in the laboratory to check for egg hatching. The eggs kept under these conditions turned a brownish color, collapsed and did not hatch; however, eggs removed from freshly cut twigs and then placed in containers where a high relative humidity was maintained showed a high percentage of hatching. Eggs removed at the same time and placed in a dry petri dish did not hatch. That the eggs of the 17-year race require a moist environment to prevent high mortality was also found by Marlatt (1907), and by Graham & Cochran (1954), both of whom noted that the drying up of broken limbs caused the eggs in the limb to shrivel and die. A similar situation has been reported for at least one other species. Beamer (1928) reported that in the species, *Melampsalta calliope* (Walk.), the eggs also withered with the wilting of the host plant.

Just after the peak of emergence over 550 live adult cicadas were captured and preserved. Slightly over 8% of these had the posterior tip of the abdomen missing and this area was covered with a yellowish-tan cake or dust-like substance. This condition, as reported by Marlatt and also by Steinhaus (1951), is characteristic of a fungus disease, *Massospora cicadina* Pk., of the 17-year periodical cicada, and presumably the condition noted here was the result of this same fungus.

Two distinct sizes were present in the adults collected. The smaller specimens, comprising 37% of the total collected, would be classified as *cassinii* (Fish). Mixed broods of these and the true *M. septendecim* commonly emerge together in the 17-year race. This has been reported for Kansas (Beamer 1931), New Jersey (Weiss 1953), and Indiana (Jacobs 1954). Alexander & Moore (1958), among others, reported differences in the biology of the two forms. The names *septendecim* and *cassinii* originally referred to the species of the 17-year cicada. The use of these names here is in accordance with past usage for the 13-year race although this application may prove erroneous when the taxonomy of both groups is completely understood.

The sex ratio, recorded on more than 500 adults, showed 48.5% females for the large form and 64.0% females for the small form. The overall sex ratio for both forms combined was 54.9% females which, when tested by a Chi square test, indicated a significant ($P=0.05$) deviation from a 50:50 ratio. Reports in the literature also show a larger proportion of females for the 17-year race. Graham & Cochran's (1954) data showed 60.6% females for the periodical cicada in Maryland. Jacobs' (1954) data, based on cast nymphal skins in Indiana, showed 70.3% for the small form and 60.5% females for the large form with an overall ratio of 64.6% females. Jacobs' data as well as those of the present study indicate that the overall proportions of females may depend upon the percentage of small forms in the population studied, as in these studies the small form showed a higher percentage of females than the large form.

Table 2.—Analysis of significance of differences in numbers of eggs laid by the periodical cicada in different species of trees.[a]

Host Plant No.	Host Plant Number[b] 1	2	3	4	5	6	7	8	9	10
1			**						*?	
2										
3					***	***	**	**		***
4										
5									*	
6										
7									*	
8										
9										
10										

[a] Stars indicate levels of significance as follows: *=5%, **=2%, and ***=1%. Blanks indicate no significant differences.
See table 1 for host plant names.

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Insecticide Tests for Tobacco Flea Beetle and Green Peach Aphid Control in Maryland Tobacco[1]

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In Southern Maryland, the tobacco flea beetle (*Epitrix hirtipennis* (Melsh.)) attacks tobacco soon after the plants are transplanted from the bed to the field. The green peach aphid (*Myzus persicae* (Sulz.)) usually attacks tobacco most severely late in the growing season when tobacco plants are large. If insecticidal control programs are to be employed for control of these two pests, they must be applied at separate times. In late applications good coverage is difficult and often results in mechanical injury to the plants.

Hofmaster (1956) reported controlling the flea beetle complex that attacks potatoes on the Eastern Shore of Virginia with preplanting soil applications of some of the chlorinated hydrocarbon insecticides. The work reported on in this paper was carried out to evaluate this method of flea beetle control and to test several materials in a separate one-spray schedule for control of the green peach aphid.

METHODS.—A superimposed split-plot design was utilized in the experiment to evaluate the control methods of tobacco flea beetle and green peach aphid. The treatments were replicated three times. There were two soil treatments for flea beetle. Dieldrin was applied to the soil at the rate of 1.5 pounds per acre and heptachlor was applied in a similar manner at the rate of 2 pounds per acre. After spraying these onto the soil surface in a water emulsion, the field was disked lightly before the tobacco plants were set. The materials evaluated for aphid control were dimethoate, Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide), phosphamidon and phorate.

Dimethoate and phosphamidon were applied at the rate of .5 pounds per acre. Thiodan was applied at 0.75 pounds per acre. The plots were treated on August 4 after the aphid population had risen to a measurable level (table 1). The sprays were applied with a high clearance sprayer using four D2-23 *Tee Jet* nozzles per row; 2% phorate granules were broadcast and watered into the plant bed 10 days before the plants were transplanted on June 12. This was done at the rate of 3 pounds per acre.

Flea beetle counts were made by counting the beetles on 50 plants per plot. The first count was made on July 9 and continued at intervals through July. Aphid infestation was determined by counting small numbers and estimating large numbers of aphids to give a reasonable estimate of aphids per plant. Twelve plants per plot were examined for each aphid count.

Table 1.—Results of insecticide tests for green peach aphid control on tobacco, 1959.

TREATMENT	AVERAGE NO. APHIDS PER PLANT ON DATE SHOWN				VALUES PER ACRE ($)	YIELDS PER ACRE (LB.)
	8/3	8/5	8/10	8/17		
Dimethoate, 0.5	209.4[a]	1.3	10.5	47.7	1,041.77	1,734
Thiodan, 0.75	143.7[a]	.9	8.1	139.7	1,148.44	1,719
Phosphamidon, 0.5	166.8[a]	1.3	17.1	56.9	1,150.33	1,835
Phorate,[b] 3	84.6	1.9	16.7	611.3	1,133.77	1,622
Untreated	181.8	2.0	14.8	374.9	1,139.33	1,668
L.S.D. at 1% level				129.2		
at 5% level	n.s.			82.6	n.s.	129

[a] Pretreatment infestation count.
[b] Granules applied to planting bed 10 days before transplanting.

On August 20 the two middle rows of the four row plots were cut, speared and hung in a curing barn. After curing, the tobacco from each plot was stripped from the stalk, graded and weighed. The dollar value of each plot was determined by applying the amount of tobacco from each grade to the current price of the respective grades.

RESULTS.—Table 1 gives the results of the pretreatment aphid counts and the counts of aphids following the application of insecticide. The analysis of variance for the August 3 count indicates that the phorate bed treatments were ineffective at that time. The data from the August 17 count were analyzed to compare the effectiveness of the treatments before harvest. Dimethoate, Thiodan and phosphamidon significantly reduced the aphid infestation until the last count, 3 days before the tobacco was cut. Analysis of variance of yield data indicates that only phosphamidon resulted in an increase in yield. None of the treatments significantly increased the dollar value of the tobacco.

Because the results of the soil treatments for flea beetles were insignificant, and the populations of flea beetles were small, the results of the flea beetle counts and yield data are not presented here.

DISCUSSION.—All three of the compounds used as foliar sprays reduced the aphid infestation until the plants were harvested. Table 1 indicates that there was a carry-over effect in the check plots. The reduction of aphids in the check plots on August 5 was probably caused by drift from the treated plots into the check plots. It is possible that if this carry-over effect or drift could have been prevented, differences in infestation, yield and value might have been more significant. Phorate bed treatments did not seem to be a promising method of carrying a systemic to the field, as the time from treatment to aphid buildup is too long, or the 10-day period from treatment to transplanting was not sufficient.

There are several possible reasons why the soil treatments for flea beetle were not effective. The population of flea beetles was small. Whether this is because a reduction in the entire experimental area was caused by the influence of the treated plots, or because the infestation was generally low is not known. It would seem that soil treatments under the conditions that existed would have to be tested on a larger scale to evaluate more accurately this method of flea beetle control.

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Occurrence and Control of Sand Wireworm in Missouri[1]

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The sand wireworm, *Horistonotus uhlerii* Horn, has been known to occur in Missouri since 1916 (Gibson). However, no extensive crop damage of economic importance has been reported. This insect is known to occur in the Carolinas, Illinois, Missouri, Arkansas, Mississippi, Florida, Texas, Georgia, and Louisiana. It may be a serious pest of cotton, corn, cowpeas, and other crops in porous, light, sandy soils that are deficient in organic content.

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In Missouri, the sand wireworm occurs in the southeast area of the State where porous, light, sandy soils are found. Several ridges of this type of soil are present in the counties of Dunklin, Scott, New Madrid, Mississippi, and Pemiscot. According to Jenkins (1955) the sand wireworm caused moderate to heavy damage to corn and some garden crops in sandy areas of south east Missouri. Kyd *et al.* in 1957 reported widely scattered fields of small corn in Dunklin and New Madrid counties had spots of one-quarter to 1 acre killed by sand wireworms. Again in 1959, Kyd *et al.* found that the sand wireworm appeared in sandy areas of southeast Missouri with small spots killed in scattered fields. Wilted or dead corn and cotton plants indicate that sand wireworms may be present in sandy knolls in fields scattered throughout the entire southeast Missouri area.

A field of corn near LaForge, Missouri, in New Madrid County, planted May 15, 1959 showed symptoms of sand wireworm damage the second week of June. Of 15 acres in the field, approximately 5 acres were injured with 3 acres severely damaged. Examination of the roots revealed three to six larvae immediately under or feeding upon the roots of each wilted corn plant. Infested corn plants ranged from 2 to 3 feet in extended height while uninfested plants ranged from 5 to 7 feet in extended height. An experiment was set up to determine whether the application of a soil insecticide would reduce the damage caused by this insect and as a result increase the corn yield.

MATERIALS AND METHODS.—For an experimental plot within the infested field, a 1-acre rectangular area of severely injured corn was selected and disked to destroy the remaining stalks.

The insecticides and rates of application per acre were as follows:

Heptachlor—emulsion, 1.5 pounds.

Hercules Powder Company Experimental Compound 5727—(Carbamic acid, methyl-,*m*-isopropylphenyl ester) emulsion, 1.13 pounds.

American Cyanamid Company Experimental Compound 18133—(*O,O*-diethyl *O*-2-pyrazinyl phosphorothioate) 10% granular material, 1.2 pounds.

Check—These plots received no treatments.

A randomized block design was used with four replications. Insecticides were applied and disked under immediately. The corn was planted on the same day, June 19. Treatments were broadcast over the old rows for plots four rows wide, spaced 38 inches apart and 100 feet long. An early maturing variety of corn (Iowa 4570) was selected for planting in an attempt to achieve ear maturity before frost. A two-row corn planter was used to drop one seed approximately every 12 inches. Several rows of corn were planted adjoining the insecticide test plots for wireworm observations.

Stand counts were made in the two center rows when the corn was approximately 3 feet high. The same two rows of corn were harvested and weighed September 27 for data on yields.

DISCUSSION AND CONCLUSIONS.—No field counts of sand wireworms were made; however, plants in the bordering observation rows were checked periodically for the presence and activity of larvae. Various sizes of wireworm larvae were found; however, most larvae appeared to be approaching maturity at the time of planting. The life cycle of the insect seemed to follow closely the observations made by Tenhet & Howe (1939). During hot and dry weather, larvae were found at depths of 15 to 20 inches. During wet, cool weather larvae were found within 2 to 3 inches of the soil surface and feeding upon the roots of the corn plants. Larvae were observed until the latter part of August.

Table 1 presents a summary of the stand counts and yields obtained with the various treatments. The stand on the plots treated with the three insecticides ranged from 50% to 69% higher than in the untreated check plots. When the corn was harvested September 27, all three insecticides gave significant increase in yield over the untreated check. However, Duncan's multiple range test failed to show any significant differences among the stand counts or yields from the plots treated with the three insecticides.

Table 1.—Effect of insecticides for sand wireworm control on stand count and corn yield. LaForge, Missouri, 1959.

TREATMENT	DOSAGE (LB./A.)	STAND[a] COUNT	YIELD (BU./A.) 15.5% MOISTURE
Am. Cyanamid 18133	1.2	209	56.4
Heptachlor	1.5	186	55.0
Hercules 5727	1.13	202	51.9
Untreated	—	125	37.7

[a] Mean of four replications.

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Mass Production of the Citrus Red Mite[1]

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In the course of studies with a virus disease of the citrus red mite (*Panonychus citri* (McG.)) (Munger *et al.* 1959, Smith *et al.* 1959), it became necessary to produce large numbers of this mite. Conditions required for rearing it continuously in the laboratory for biological and toxicological studies had been established (Munger 1956, Munger *et al.* 1960), but they were not designed for large-scale production. A method for mass production is described here which may be useful to other workers.

It was found that a stream of air will dislodge mites from host lemons without damaging the eggs. For transferring egg-laying mites from one set of lemons to another, frames with wire stretched across in both directions to form squares are used for holding the lemons (fig. 1). These frames are 18 inches square and constructed of $1\frac{1}{4}$ by $\frac{3}{4}$ inch pine. The wire is radio-antenna cable, soldered at the intersections, which are $1\frac{3}{4}$ inches apart. Each frame holds 32 lemons about 2 inches in diameter. Dark green lemons are used because they will support higher populations than those that have broken color.

An infesting tower (fig. 2) consists of a wooden frame 32 by 22 by $23\frac{1}{2}$ inches, made of the same stock as the frames to hold the lemons, with a funnel suspended inside. The funnel is a 4-sided pyramid $19\frac{1}{2}$ inches square at the top with an opening 10 inches square at the bottom. It is constructed of smooth white cardboard made glossy with white lacquer.

To obtain eggs, lemons are placed on tissue paper laid over the wire on the frame, which is set 5 inches below the bottom of the funnel, and another frame, without tissue paper, bearing lemons infested with mature mites, is set on top of the tower with a wooden collar 5 inches tall around the lemons. Compressed air

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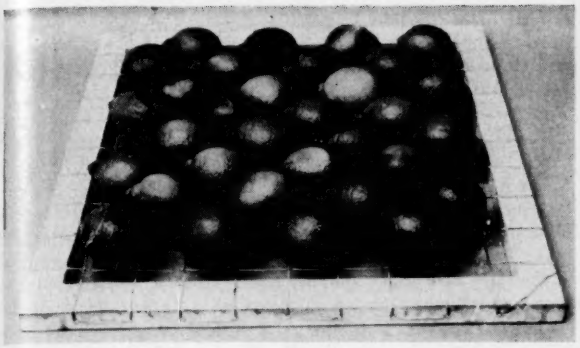

FIG. 1.—Wire frame holding 32 lemons.

from a nozzle having jets with narrow elongated openings in line with each other to produce a brush effect is applied to dislodge the mites. An empty frame is then fitted over the upper side of the lemons, and while the two frames are held together, they are inverted so that the mites can be blown from the other side. The mites fall to the paper and the lemons on the frame at the bottom; those falling on the paper soon find their way to the fruit The mites are allowed to oviposit for about 24 hours and are then transferred to another frame of fresh lemons. This egg-laying population is used repeatedly and is augmented as needed from laboratory stocks.

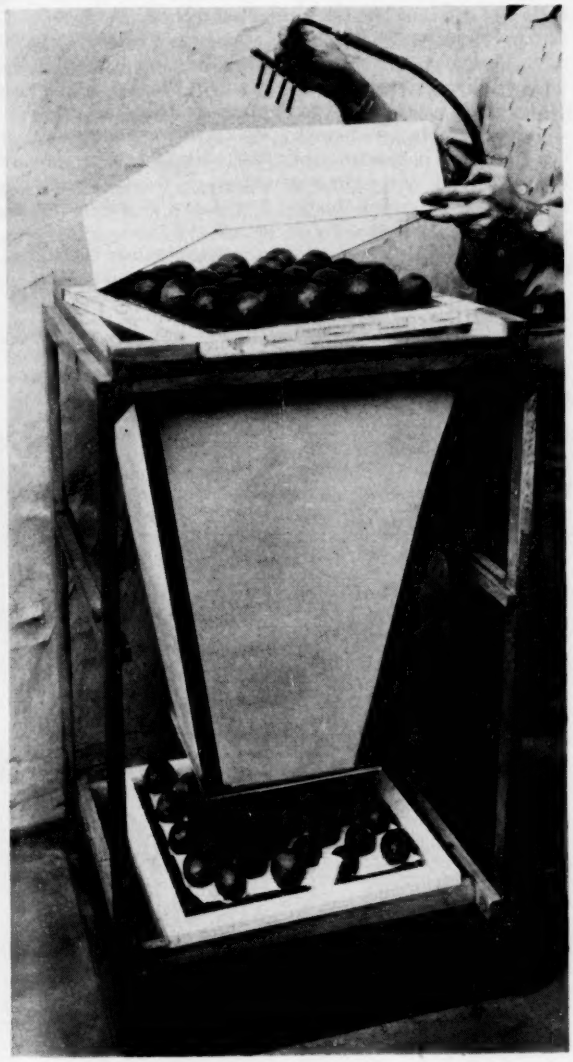

FIG. 2.—Infesting tower with lemons on frames.

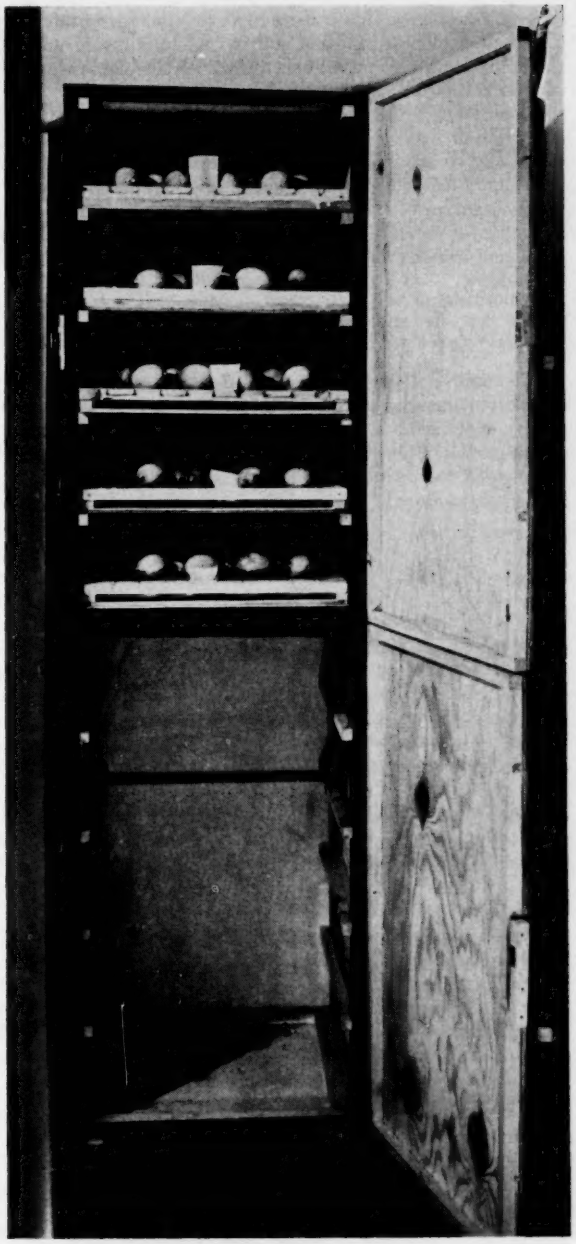

FIG. 3.—Lemons bearing eggs stored on frames in muslin-covered case for development of the mites.

Between transfers the surface of the funnel is rubbed with a flannelette cloth impregnated with talcum powder. The rubbing removes webbing left by straggler mites. Other mites tend to cling to the webbing; the talc lubricates the surface and prevents the mites from sticking. To remove any remaining mites, webbing, and other debris, the lemons are washed with a kitchen-sink sprayer attached to a hose. The frames are inverted as described earlier and the other side is washed. This process does not remove the eggs. The lemons are then placed in a muslin-

covered case (fig. 3) in a rearing room for the mites to develop. The limited ovipositing time and the washing insure mites of about the same age and provide a clean surface upon which the new generation may feed.

When the lemons lose their attractiveness through feeding damage and age, the mature mites are transferred to fresh lemons by means of rubber collars (Munger *et al.* 1960). The new lemons attract mites that would otherwise wander away.

To obtain an estimate of mite yield, 5 lemons were selected at random from each of 3 typical frames of 32 lemons. Counts per lemon were as follows:

	Range	*Average*
Ovipositing mites	168– 311	255
Eggs deposited in 24 hours	1,186–3,327	1,858
Females developed to maturity	279–1,036	522

The number of eggs laid on a given set of lemons varies considerably, depending on the number and age of the females, the quality of the lemons, and the time allowed for oviposition. To obtain maximum yields of mature mites, the eggs should be limited to the number that the feeding surface of the lemon can support to maturity.

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Preliminary Evaluation of Lindane and Heptachlor Crystals for Protection of Crated Furniture Against Insect Damage

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Insect damage to crated furniture during storage is a problem often encountered by the Armed Forces and commercial storage warehouses. In cooperation with the Armed Forces Pest Control Board tests were initiated at this laboratory to evaluate the use of lindane in protecting crated furniture against insect damage. Lindane and heptachlor crystals were applied at the rate of 5 grams of insecticide per cubic foot to large containers approximately the size of crates used by the Military to store and ship furniture. The results of these studies are reported herein.

Laudani (1958) reported that loose lindane crystals at 5 grams per cubic foot protected boxed woolens against insect damage for 3 years or longer. An objection to the use of loose crystals led to additional studies reported by Laudani *et al.* (1959) in which the crystals were adhered to cardboard with the aid of a fast-drying adhesive, and introduced into the storage containers in this form. The treated cardboards were designated as "lindane boards." The present study compares the effectiveness of lindane and heptachlor "boards" with bagged lindane and heptachlor crystals applied in furniture crates having a volume more than 40 times greater than the storage containers used in the previous studies.

MATERIALS AND METHODS.—Five large wooden crates, 88½ by 46 by 73¾ inches and having a volume of 174 cubic feet each, were lined with aluminum foil-laminated kraft paper and made as airtight as possible. Caged test insects were suspended from wires strung at 1-, 3-, and 5-foot levels, and portholes were cut near these levels to facilitate insertion and removal of the cages. In one crate 870 grams of loose lindane crystals (5 grams of lindane per cubic foot) were divided into four equal lots and placed in loosely woven muslin bags in the upper four corners of the crate. In a second crate lindane crystals were adhered to paper at the rate of 30 grams per square foot of paper surface. To obtain the desired 5 grams of lindane per cubic foot, 29 square feet of treated paper were fastened to the ceiling and walls of the crate. A third crate contained loose heptachlor crystals applied at the same rate as the lindane. The heptachlor was divided into four equal lots and placed in bags similar to those used for the lindane treatment. The adhered heptachlor crystals covered a larger surface area than did the adhered lindane crystals. The heptachlor crystals were very fine and fluffy and the same weight could not be applied to an equal area; therefore kraft paper was treated at the rate of 20 grams per square foot. At this rate 43.5 square feet of treated paper covered with 870 grams of crystals were required to give the desired 5 grams of heptachlor per cubic foot. The treated paper was fastened to the ceiling and walls of the crate. The fifth crate was used as an untreated check.

Flat, envelope-type cages containing black carpet beetle larvae, *Attagenus piceus* (Oliv.), were inserted through the portholes and suspended on the wires. Four replications of 10 insects each were removed from each level after exposures of 14, 28, and 42 days. The insects were transferred to clean petri dishes and mortality readings were made initially and at the end of 7, 14, and 21 days' post exposure. When the treatments had aged 42 days, additional insects were placed in the crates for 14-, 28-, and 42-day exposures. The crates were stored in an unheated warehouse-type area in which the temperatures ranged from a low of 48° to a high of 88° F. during the first 6-week period and from 34° to 74° F. during the second 6-week period.

RESULTS AND DISCUSSION.—The results obtained with the adhered and bagged crystals of both insecticides at various ex-

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[2] This is one of the field stations of the Stored-Product Insects Branch, Market Quality Research Division, Agricultural Marketing Service, U. S. Department of Agriculture.

Table 1.—Percentages of dead plus moribund black carpet beetle larvae after exposure to vapors from lindane and heptachlor crystals applied in 174-cubic feet containers at the rate of 5 grams of insecticide per cubic foot.

	Exposure (days)					
	14		28		42	
	Post Exposure (days)					
Insecticide	0	21	0	21	0	21
	Insects exposed to freshly applied treatments					
Adhered crystals:						
Lindane	99	100	100	100	100	100
Heptachlor	100	100	100	100	100	100
Bagged crystals:						
Lindane	30	30	56	65	70	89
Heptachlor	34	85	63	100	88	100
None (check)	2	2	0	0	1	1
	Insects exposed to treatments 42 days old					
Adhered crystals:						
Lindane	99	100	100	100	100	100
Heptachlor	97	100	100	100	100	100
Bagged crystals:						
Lindane	11	19	61	62	89	90
Heptachlor	45	78	100	100	97	100
None (check)	0	0	0	0	0	0

posures are shown in table 1. On the basis of the percentages of dead plus moribund insects, the adhered crystals were more toxic than the bagged crystals, the insecticides were equally effective in the adhered crystal form, and heptachlor was more effective than lindane in the bagged form. This was true regardless of whether the insects were introduced into the crates when the treatments were freshly applied or after they had aged for 42 days.

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An Improved Trap for Deciduous Tree Fruit Flies[1]

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A trap consisting of a container, such as a half-pint fruit jar, to hold the attractant and a sticky board superimposed on the lid to trap the insects attracted to it has proved highly effective in capturing apple maggot (*Rhagoletis pomonella* (Walsh)), cherry fruit fly (*R. cingulata* (Loew)), and black cherry fruit fly (*R. fausta* (O.S.)) adults. The board is of $\frac{1}{8}$-inch tempered masonite, 6×8 inches, painted with yellow enamel. It is made sticky by a coating of Deadline or other material that retains its tackiness. The simpler forms of such traps allow the attractant to become diluted during rainy weather, so that their efficiency is reduced.

A specially designed trap (fig. 1) reduces the amount of rain entering the bait container. A right angle strip of sheet copper $\frac{1}{2}$ by $\frac{1}{2}$ by $2\frac{3}{4}$ inches is soldered across the top of the rim of the jar lid band and the board bolted to it. The protection against rain is similar to a double roof and consists of two pieces of sheet copper bolted to each side of the board above the lid band. The lower roof is $3\frac{1}{2}$ inches wide and 2 inches deep plus a $\frac{1}{2}$-inch turnup tab to bolt to the board. An opening 1 inch square by $\frac{3}{4}$ inch deep is cut next to the board to allow the odor of the attractant to escape near the center of the board as well as at the edges. The upper roof, also of sheet copper, is $1\frac{1}{2}$ inches square plus a turnup tab $\frac{1}{2}$ by $3\frac{1}{2}$ inches that is secured to the board by the same bolts as the lower roof.

The angle of the roof may be altered by bending it to allow maximum lure diffusion and a minimum of rain to enter.

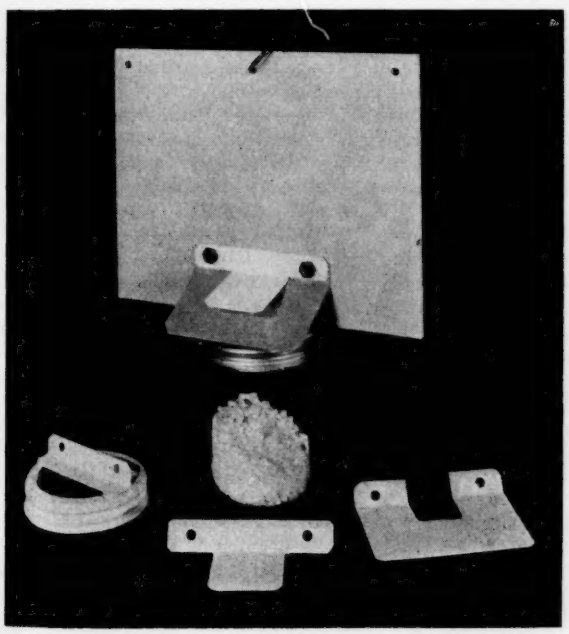

FIG. 1. Improved trap for deciduous tree fruit flies.

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Combinations of Acaricides with Complementary Activity[1]

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As many acaricides are highly effective only against certain stages in the life cycle of tetranychid mites, pairs of acaricides that complement each other to destroy all stages can be expected to be more effective than either acaricide used alone. A number of such pairs were accordingly compared with the same materials used alone against the two-spotted spider mite, *Tetranychus telarius* (L.), on scarlet runner bean in the greenhouse. The acaricides were:

Tedion®.—2,4,4′,5-tetrachlorodiphenyl sulphone, 1 lb. active per imperial gal., emulsifiable concentrate (Niagara Brand Chemicals). Kills eggs and young mites; also females exposed for some time lay sterile eggs (Batth & Davidson 1959); has long residual activity.

Phosdrin®.—1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate 60% technical, 12.32 lb. active per imperial gal., miscible liquid (Shell Oil). Kills only motile stages at practical dosages; has very short residual activity.

Guthion®.—*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3-(4*H*)-ylmethyl) phosphorodithioate, 25% wettable powder (Chemagro Corporation). Kills only motile stages; has good residual activity.

Polybutene.—Indopol® L-100 (Amoco Chemicals Corporation), prepared from a 75% emulsion containing 3% emulsifier. Kills both motile stages and eggs (Fisher 1959); has some residual activity.

The sprays were applied to the point of run-off on established populations containing all stages of the laboratory strain of mite, which previous tests had shown to be resistant in varying extent to the phosphates parathion, ethion, and dimethoate.

Treatments and results are shown in table 1. At the rates used, none of the materials applied singly, except polybutene, gave very high mortality. Although the Tedion would probably have given a still further decrease in the population after the 14-day period, for many of the survivors would lay sterile eggs, the former continued to feed and produce injury. Where Tedion was combined with any of the other materials the mortality was greatly increased. The combination of Tedion and polybutene was especially effective. The high mortalities from Tedion with Phosdrin and especially with Guthion are particularly important, for the mite had evidently developed considerable cross-resistance to these two phosphates.

At present, the addition of a specific acaricide, such as Tedion, to a phosphate applied primarily as an insecticide is usually recommended in Ontario orchards only after the mites become phosphate-resistant. In view of the superior control obtained in these tests, the earlier use of such combinations may be desirable not only because of the immediate increase in effectiveness but also because the development of resistance might be delayed.

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Table 1.—Control of two-spotted spider mites on scarlet runner bean by combinations of acaricides in the greenhouse.

Materials per 100 Imperial Gal.	Percentage Mortality[a] After		
	2 Days	7 Days	14 Days
Tedion 0.5 pt. 10% E.C.	3	25	55[b]
Indopol L-100 1.3 gal. 75% E.C.	97	78	64[c]
Indopol L-100 1.3 gal. 75% E.C. +Tedion 0.5 pt. 10% E.C.	98	100	100[d]
Phosdrin 2.5 fl. oz. 100% E.C.	85	17	17[b]
Phosdrin 2.5 fl. oz. 100% E.C. +Tedion 0.5 pt. 10% E.C.	89	96	87[d]
Guthion 1.25 lb. 25% W.P.	55	40	17[b]
Guthion 1.25 lb. 25% W.P. +Tedion 0.5 pt. 10% E.C.	93	98	98[d]
Check	4	6	9

[a] Based on numbers of live and dead mites at time of examination.
[b] Severe feeding injury at 14 days.
[c] Feeding injury localized.
[d] No live young; no eggs hatched at 14 days.

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Malathion Against Lead Cable Borers[1]

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The lead cable borer, *Scobicia declivis* (Lec.), is a bostrichid beetle which develops in dead parts of trees. In the Fresno, California, area the adults are on the wing beginning in March and some may be found until late in July. They are attracted to the products of fermentation, including ethyl alcohol, that occur in wine, brandy, and fermenting dried fruits including raisins. Their habit of eating circular holes in the laminated paper covers under which stacks of raisins are stored for fumigation and protection from re-infestation led to attempts to prevent such damage. Holes in the paper covers allow leakage during fumigation and provide places through which other species of insects may enter.

Both laboratory and field experiments were made. Field-collected beetles were confined in the laboratory on sprayed sheets of 1′×2′ laminated paper. Rather small numbers of beetles were available. Sprays were made by mixing 25% malathion wettable powder in water at the rate of 4 or 5 pounds of malathion in 100 gallons. Other formulations were made with malathion emulsifiable concentrate containing 5 pounds of malathion per gallon. The concentrate was used at the rate of 1 or 2 gallons per 100 gallons of water to make finished sprays containing 5 and 10 pounds of malathion per 100 gallons. Each spray was atomized onto the test papers at the rate of 2.6 gallons per 1,000 square feet. Intended deposits were 47 and 59 mg. per square foot from the wettable powder and 59 and 118 mg. from the emulsion.

Exposures of beetles were for various periods up to 1 hour. Deposits were tested soon after they had dried and when they were from 1 to 10 days old. Beetles confined on fresh residues of the wettable powder sprays died rather rapidly. They showed paralysis after 24 hours and all were dead after 72 hours. Deposits 2 days old brought death in 48 hours to beetles exposed for 1 to 5 minutes. Ten-day-old deposits produced poor kills. Residues of the emulsions were not so effective as those of the wettable powders.

A trombone-type hand sprayer was used to apply a formulation that contained 20 pounds of 25% malathion wettable powder per 100 gallons of water. Approximately 1¾ gallons were required to treat a stack end, an area of about 500 square feet. The intended deposit was 80 mg. of malathion per square foot. The sprays were applied April 24, May 7, and May 21. Beetle holes were patched prior to each spray application. Entrance holes were counted at intervals from April 27 to June 5. An untreated area of 500 square feet was penetrated by 55 holes. The sprayed area of equal size contained only eight penetrations.

A wettable powder spray was applied to nine paper-covered raisin stacks on July 3, using a 100-gallon power sprayer. One stack was left unsprayed. Lead cable borer holes were repaired with paper patches before the spraying was started. The spray was prepared by adding 13 pounds of 25% malathion wettable powder to 100 gallons of water. The exposed area of the nine stacks was 20,700 square feet and the estimated deposit was 70 mg. of malathion per square foot. It was found that spraying in vertical panels was most efficient.

Protection of the sprayed stacks was good in comparison with the untreated check. The effectiveness of the deposits decreased with aging. Average protection compared with the check stack was as follows July 13, 86%; July 18, 77%; July 25, 72%; July 30, 64%.

[1] Accepted for publication May 25, 1960.
[2] The tests reported in this note were conducted by Dwight F. Barnes (deceased), Samuel B. Stagg (resigned June 1958), and George W. Riley, associate technical director of Dried Fruit Association of California at the time tests were made.

Escape of Methyl Bromide Through Wallboards[1]

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A cubical box 4′×4′×4′, made of plywood with one side open for attachment of test panels, was used to obtain information on the escape of methyl bromide through four types of wallboard. The box was well painted on the inside and its tightness was checked occasionally by covering the open side with a piece of Panelwood lined with one thickness of impermeable 4-mil polyethylene sheeting. A square frame of 1-inch strap iron was applied to the outer edges of a test panel and three screw clamps on each of the four sides held the panel in place against a gasket on the open side of the plywood box.

Methyl bromide, at a dosage rate of 1 pound per 1,000 cubic feet, was introduced through an opening in the top of the chamber. A small fan kept the fumigant-air mixture stirred while volatilization took place. Samples were drawn from near the top and the bottom of the box 2, 6, and 24 hours after the fumigant was applied. Analyses were made by the Volhard method.

The four types of wallboard—Masonite Panelwood, standard Pressdwood, tempered[3] Pressdwood, and tempered Duolux—were each tested three times before they received any treatment. Then each wallboard was painted with Thompson's Water Seal paint, dried, and tested three times. A coat of Fuller's Ful-Alumina asphalt-aluminum emulsion was then put on, and each sample was again tested three times.

The chamber closed with the polyethylene-lined square of Panelwood retained 73.8% of the fumigant at the 24-hour time-point. Gas retention with the unpainted wallboards was as follows: Panelwood 8.6%, standard Pressdwood 20%, tempered Pressdwood 46.9%, and tempered Duolux 50.6%. The water-seal paint did not improve these results. After the aluminum-asphalt emulsion was applied the retention figures were as fol-

[1] Trade names are used for identification only. No endorsement of special brands by the U. S. Department of Agriculture is made. Accepted for publication May 25, 1960.
[2] The tests reported in this note were conducted by Dwight Barnes (deceased) and Samuel B. Stagg (resigned June 1958).
[3] Made more dense by heavier pressure.

lows: Panelwood 37.5%, standard Pressdwood 71.9%, tempered Pressdwood 60.6%, and tempered Duolux 77.5%. Even though the painted Panelwood retained the least gas there was enough methyl bromide in the box to kill any test insects associated with raisins.

The cheapest satisfactory material (unless prices have changed substantially) is Masonite Panelwood or its equivalent with one or even two coats of an asphalt-aluminum emulsion. Unpainted Panelwood or its equal is not satisfactory because it retains little or no methyl bromide during 24-hour fumigations.

Experiments to Exclude *Drosophila* from Dried Fruit[1]

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This report brings together (1) the results of work done by Dwight F. Barnes (deceased), Erwin F. Sawall, Jr., and George W. Reilly in 1955 on the use of fans at dehydrators to protect fresh grapes from *Drosophila* spp., and (2) tests of open-weave fabrics for the same purpose by Lee A. Wood, Jr., and the writer in 1954. The participants not listed as authors are no longer stationed at the Fresno Laboratory, but credit for most of the work belongs to them.

Most raisins made in California are sun-dried but a small percentage of the 200,000-ton crop is dehydrated to make golden-bleached raisins. Although raisin grapes on the vines in the San Joaquin Valley may become infested by *Drosophila* spp., chiefly *D. melanogaster* Meigen, swarming of adults occurs chiefly on grapes stacked in picking boxes in vineyards or on or near loading docks at dehydration plants. In order to avoid infestation handlers need improved means for protecting the fruit before it is processed into golden-bleached raisins, which includes dipping in water containing lye, washing, sulfuring, and dehydrating. Evidence of infestation on the finished golden raisins has received increasing attention from food inspectors in recent years. Pileup of freshly harvested grapes, either in vineyards or at dehydrators, should be avoided, but even with good planning a supply of fruit must be held overnight in order to get the plant started in the morning before new deliveries begin to arrive from the vineyards.

Two three-box stacks of cull grapes containing crushed, rotten, and moldy berries were set up on a loading dock for each test. One stack was left as a check; the other was air-washed by means of electric fans according to different schedules. The bunches of grapes to be used in the tests were selected before the flies had a chance to settle on them or were freed of most of the insects by holding each bunch in front of a fan. The fans were three-bladed, 17-inch fans having a capacity of 4,500 cubic feet per minute 7 inches in front of the blades. Hand anemometers were used to measure air movements. Some flies reached the grapes when a single fan was directed against a stack from a distance of 12 feet, but the reduction compared with the check stack population was 89%. Reduction was 97% when two fans were used to air-wash a stack on all sides, the air being directed diagonally against corners of the stack so that each fan air-washed one side and one end of the boxes. Speed of the air was from 3 to 6 or 8 miles per hour. Exposure periods were 20 hours or more, beginning in midafternoon.

The reduction in flies was ascertained by the following method. Flies on the grapes were immobilized by spraying the fruit in the treated and untreated stacks of boxes with pyrethrum spray. The grapes were then immersed in water and the flies that floated were recorded. When a single fan was used the average populations of flies per 50-pound box were: air-washed 142; untreated 1,258. Two fans gave the following protection: air-washed 33 flies per box; untreated 1,327.

The experiment with closely woven fabric as protection for fresh fruit was done in heavy paperboard drums, laid on their side and open at both ends except for a covering of 40-mesh cheesecloth. Placed in the shade out-of-doors, these rearing chambers satisfied the requirements of the flies for reduced light, moderate air movement, and favorably cool morning and evening temperatures. There was no activity at night. A large population of flies, in this case *Drosophila simulans* Sturtevant, a species close to *D. melanogaster*, was reared on crushed grapes or canned pumpkin.

Uninfested dishes of attractive food were introduced into the chambers. Each dish was covered with a piece of fabric to be tested; either tobacco shade cloth, 10 threads per inch, or 40-mesh cheesecloth. The tobacco shade cloth was tested plain (single and double thickness) and also after the pieces were dipped in pyrethrum or methoxychlor formulations. The pyrethrum stock emulsion contained 1.18% pyrethrins, 11.84% piperonyl butoxide, 14.81% emulsifier, and 72.17% deodorized kerosene. This stock was used in water at 5% strength. The methoxychlor emulsion contained 24% technical methoxychlor, 63% methylated naphthalenes, 10% dimethyl phthalate, and 3% inert ingredients. Two strengths, 5% and 2%, were used in making the dips.

All treated and untreated shade-cloth barriers, whether in one or two layers, permitted flies to enter within 24 hours and large numbers gained entrance within 48 hours. No *Drosophila* entered dishes of food protected by 40-mesh cheesecloth and no larvae developed in the food.

[1] Accepted for publication May 25, 1960.

Malathion Sprays for Reducing Dried-Fruit Packing House Infestations[1]

PEREZ SIMMONS[2]

Areas in dried-fruit packing plants where cases of packed goods are stacked while awaiting shipment usually are kept clean. However, these areas may be close to storage places for unprocessed dried fruits, some of which may be newly received and not yet fumigated. Such a situation may be an infestation hazard to commodities stored on shipping floors. With this hazard in mind a survey was made extending through parts of three seasons, 1955 to 1957.

Traps devised by Mr. George Riley were distributed. They were made of two 7-inch paper pie plates, with the concave sides facing, joined together at a point where the rims touched with a single staple. Four holes were punched in the side of the lower plate, near the bottom, to serve as entrances for crawling insects. A handful of raisins was the bait used in all the traps except one, in which dried peaches were tried. Collections were made twice weekly as a rule. Captured insects were separated from the baits by sifting, and the baits were then replaced in the trap.

A survey made in eight plants in 1955, using 43 traps, showed that there was a control problem in four of them. In plant 1 the lower 4 feet of wall and a 2-foot strip of adjacent floor was sprayed with a formulation containing malathion at a concentration of 5 pounds in 100 gallons of water. This and subsequent spray formulations were prepared from a 25% malathion wettable powder. When dry the spray left a visible deposit. A second application was made on October 11. The insect counts, although never high, dropped abruptly after the sprays were applied.

Infestations were measured in 1956 by trapping in eight plants in the San Joaquin Valley and San Jose areas of California. Seventy traps were put out on shipping floors. In plant 8 all open spaces on the shipping floor were sprayed on September 14

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[2] The tests reported in this note were conducted by Dwight F. Barnes (deceased), Samuel B. Stagg (resigned June 1958), Erwin Sawall (resigned January 1956), and George W. Riley, associate technical director of Dried Fruit Association of California at the time tests were made.

with a formulation containing 3 pounds of malathion in 100 gallons. A sharp reduction in the insect population followed.

Plant 9 was sprayed on August 17 with a formulation containing 5 pounds of malathion per 100 gallons. The sprayed area was 8 feet wide (4 feet around the perimeter of the floor and 4 feet up the walls) and totaled 2,880 square feet. Three and three-quarters gallons of spray were applied, producing a calculated deposit of 29.7 milligrams of malathion per square foot. The same areas were sprayed again on September 4 and on October 2. Trapping indicated marked improvement in the insect situation after the first spraying, but there was an invasion of larvae of the raisin moth, *Ephestia figulilella* Greg., during the week following the last spraying.

The shipping floor of plant 10 was sprayed on September 14 and again on November 9 with a formulation containing 3 pounds of malathion in 100 gallons of water. Comparatively large catches of insects were taken in the shipping area early in September. This part of the plant was near the raisin stemmer and there was no barrier between. A total of 127 saw-toothed grain beetles, *Oryzaephilus surinamensis* (L.), were collected on September 11 and 79 on September 14. The first collection after spraying (September 18) totaled 18 beetles.

Trapping in 1957 was confined to 14 traps on the shipping floor of plant 15. The owner sprayed four times at monthly intervals. Insect catches for 5 months, from July 5 to December 16, totaled only 96 insects. Catches included the dominant *Oryzaephilus surinamensis*, *Carpophilus* spp., *Drosophila* sp., dermestids, *Tribolium* spp., *Ephestia figulilella*, *Blapstinus* sp., psocids, silverfish, *Cephalonomia* sp., and ants.

A New Technique for Shipment of Natural Enemies of Insects[1]

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In foreign exploration work the biggest problem confronting the entomologist is likely to be the satisfactory survival of parasites or predators en route home. This has been greatly alleviated by the development of air transport; still, certain species, which for one reason or another are preferably shipped as active adults, have exhibited high mortalities during transit. Often adult parasites or predators are more easily obtained by the collector than are the immature forms. Of course, other species, which can be shipped as larvae or pupae inside the host insect and which do not emerge en route, do not present as great a problem. Quarantine restrictions often preclude the inclusion of host-plant material in shipments, and in the case of external parasites this means increased exposure during transit. Such exposure is particularly adverse to small external parasites of diaspine scale insects such as *Aphytis* and *Thysanus*. The junior author had particularly poor results with shipments of both adult and immature *Aphytis* spp. from the Orient to California, especially when parasitized scales on the host plant could not be included.

There are two main problems during air shipment—one is to provide food for the adult parasites or predators which are included or which emerge en route; the other is to minimize exposure to extremes of temperature and humidity which may be encountered. With many parasites and predators, honey applied in thin streaks is a standard adult food. However, its use under shipment conditions is impractical with small parasites which easily become mired in the honey, or in cases where debris or host material is included which sticks in the honey and makes it inaccessible as food. Hence, attempts to devise better techniques for shipment of beneficial insects were carried out, and although these have resulted in an improved method, various ramifications need further work. It seems advisable, however, to review the results obtained thus far because they may be of immediate value to other investigators engaged in similar work.

FOOD SUPPLEMENTS.—Preliminary studies utilizing adult parasites of the genus *Aphytis* as test organisms indicated that a solid or semisolid material having a high moisture content would be a more suitable form of nutrient during transport than pure bee honey, the food supplement generally employed, particularly in cases where large numbers of parasites are to be confined in comparatively small, sealed containers.

The use of pure honey as a food medium has several disadvantages. Inasmuch as honey tends to absorb moisture under certain conditions, its consistency may change from a thick syrup to a thin adhesive film which in transit may spread over large areas of the shipping container, thereby ensnaring the parasites. Honey is also subject to spoilage when exposed to hot, humid conditions.

Earlier trials in which small amounts of honey were covered with thin films of various waxes (in order to prevent small parasites from becoming stuck) were found to be satisfactory for laboratory use but were deemed inadequate for shipment, inasmuch as the honey flowed and the wax films tended to rupture when subjected to sudden movement or prolonged periods in an inverted position.

Several media, including honey-agar mixtures and various dextrose agars, were considered by the authors and temporarily rejected because of the complexity of formulation, procedures involved, and other reasons.

The medium finally selected for further trial consisted of a gel composed of 125 ml. of orange juice, 63 ml. of pectin (MCP brand), and 250 ml. of granulated cane sugar, prepared in a manner similar to that employed in making household jelly, according to the instructions furnished by the processors of the pectin. (Water or other liquids may be substituted for the orange juice in this mixture, and the authors contemplate the use of several supplements in future formulations.) Early trials utilizing this medium indicated that adult *Aphytis* spp. could be held, in the absence of host material, for periods of time equaling or surpassing that of honey-fed parasites. Sometimes the surface of this gel will tend to be slightly sticky, so in order to preclude the possibility of parasites becoming stuck, a thin wax film of the type already tried with honey was used. This is applied as a wax in solution[2] which evaporates out to leave a thin, dry, firm film over the gel through which the parasites readily feed.

SHIPPING CONTAINERS.—With the knowledge from experience that parasites in transit may be subjected to low and sometimes lethal temperatures such as occur in high altitude flights in unheated cargo planes, and conversely to extremely high temperatures which may occur when cargo is temporarily stored or held on the air-strip in transfer operations, a compact, well-insulated shipping container was required that would afford protection against adverse climatic conditions for a period of time. Small commercial vacuum bottles of the type used for cold or hot drinks came to mind as a logical choice for trial. The ones selected for use in the first shipping tests were one-pint vacuum bottle fillers of the type commonly used in thermos bottles, measuring approximately $2\frac{3}{4}\times 8$ inches and weighing 6 ounces (including the cork stopper). Preliminary tests showed that *Aphytis* adults, when packaged in these vacuum bottles, could withstand outside temperatures of $-4°$ F. ($-20°$ C.) for up to 4 hours' duration, and sublethal outside temperatures (near or below freezing) for 8 hours or longer with no ill effects. Shipping tests were then arranged.

Several days prior to the intended shipping date, a fresh mixture of the food supplement was prepared and used to coat the

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[2] Acknowledgment is made to Dr. A. F. Kalmar, Chief Chemist of the Research Division of the Food Machinery and Chemical Corporation Packing Equipment Division at Riverside, for his advice and for making available several types of solvent waxes for these experiments. The wax used in this instance is a Food Machinery Corporation product known as "Flavorseal," which is usually used as a protective film over fruits.

inside surface of the vacuum bottles. Only a thin film is desired or its weight may tend to separate it from the sides of the container. The media must be poured into the containers while it is still hot and in a fluid state, inasmuch as it tends to gel rapidly. Two bottles were completely coated inside with a thin film ($\frac{1}{16}$ to $\frac{1}{8}$ inch thick) of the prepared gel by rotating the bottles in a horizontal position for one full turn and then pouring out the excess liquid. Another two bottles were partially covered inside with an "agar-type" slant ranging to one-fourth inch (or more) in thickness. The bottles were left unsealed for a few days (preferably 4 to 6 days) to allow the medium to thoroughly "jell" or solidify, and to evaporate excess surface moisture, and then the gel was thoroughly coated with a thin film of liquid wax (to prevent the parasites from becoming stuck in the medium) and again set aside for a few hours to allow the wax solvent to evaporate completely.

Preparatory to shipment, an estimated 1400 to 1600 adult parasites (*Aphytis lingnanensis* Comp.) were placed in each thermos bottle and sealed with cork stoppers. The bottles were then placed in individual close-fitting corrugated cardboard containers, which in turn were placed in larger shipping cartons, measuring approximately 6 inches square by 12 inches in length, and padded on all sides with 2 inches of crushed paper toweling and the cartons sealed with a gummed tape. On April 18, 1960, two cartons, one containing a vacuum bottle with the "agar-type" slant and one with a vacuum bottle having the overall thin gel coverage, were sent by air from Riverside, California, to D. W. Jones, Insect Identification and Parasite Introduction Research Branch, U. S. Department of Agriculture, Moorestown, New Jersey. Replicate units were held at a constant temperature of 80±1° F. in the insectary at Riverside. Upon receipt of the material in Moorestown, and without being opened, the two cartons were shipped back to Riverside arriving on April 21, 1960, having completed two flights of about 2800 to 3000 miles each in a 3-day period.

After their return to Riverside, the cartons and bottles were opened and a count was made of the live parasites in these as well as in the replicate units held in the insectary with results as follows:

	Vacuum Bottle Designation	*No. Live Parasites*
Units Shipped	1. Overall film coverage of gel $\frac{1}{16}$ to $\frac{1}{8}$ inch thick	1,298
	2. "Agar-type" slant of gel to $\frac{1}{4}$ inch thick	977
Units Held	3. Overall film coverage of gel $\frac{1}{16}$ to $\frac{1}{8}$ inch thick (check for #1)	1,486
	4. "Agar-type" slant of gel to $\frac{1}{4}$ inch thick (check for #2)	1,274

A careful examination was made of the contents of each vacuum bottle at this time and the following observations were noted. Vacuum bottles Nos. 1 and 3 (overall film coverage) and 4 ("agar-type" slant) contained not more than 100 dead parasites, some of which adhered to the lining of the bottles. The gel in vacuum bottle No. 2 ("agar-type" slant) had become detached in shipment and apparently had rotated within the bottle leaving a thin sticky film over much of the surface which trapped several hundred parasites. Some condensation occurred in this bottle, inasmuch as the unwaxed undersurface of the gel was exposed when it became detached from the lining of the bottle. Because of the poor condition of the contents of bottle No. 2 following transcontinental shipment, the use of an "agar-type" gel slant for shipping purposes is not recommended.

From these trials it would appear that the use of vacuum bottles, properly prepared and packaged and utilizing in a similar manner a gel food supplement of the nature herein described (applicable to the species of insects involved), would insure the successful shipment of many species of beneficial insects on a worldwide basis under most conceivable conditions. One great advantage of this method is that food is continuously available underfoot. The relative humidity within the vacuum bottles may probably be regulated at desired levels by varying the amount of sugar in the medium according to the method of Holloway (1939).

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Adult Longevity as a Sensitive Criterion of Radiation-Induced Damage When 24-Hour *Habrobracon* (Hymenoptera) Embryos Are X-Rayed[1]

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Some radiation effects upon *Habrobracon* previously have been measured on the basis of: (1) differential radiosensitivity of eggs (Whiting 1939, 1940, 1945a,b); (2) developmental effects after radiation of eggs (Dent & Amy 1950, Amy 1955); (3) adult performance as measured by fecundity and fertility after radiation at a particular developmental stage (Clark & Kelley 1950, Grosch & Sullivan 1954, Erdman 1960); (4) increased longevity after adult radiation (Sullivan & Grosch 1953, Grosch 1956); (5) polidy (Clark & Mitchell 1951, 1952, Clark 1957); and (6) types of dominant lethality (von Borstel & Rekemeyer 1959). The results from these experiments do not indicate that decreased longevity is a manifestation of radiation damage when a developmental life cycle stage is radiated. The present paper presents evidence that adult longevity of *Habrobracon* is a sensitive criterion of radiation damage compared with other parameters when the wasps are x-rayed as 24-hour-old embryos.

As part of a radiobiological study of induced sterility, 24-hour-old embryos of *Habrobracon juglandis* (Ashmead), a wasp ectoparasitic on larvae of *Ephestia kuehniella* (Zeller) were given whole body x-ray delivered at 600r per min. by a 250 Kv generator. Experimental conditions were maintained at 30 C.° except during irradiation which was performed at room temperature. In order to obtain homogeneous samples of parthenogenetically produced males, only virgin females were x-rayed as 24-hour-old embryos and were allowed to mature. Their egg development, other than hatchability, was not scored; however, experiments with other life cycle stages indicate that irradiated gametes although reduced in numbers, have a very high capacity to mature into adults (Erdman, unpublished data). Egg viability was high at all times.

Data on egg production from mothers x-rayed as embryos from 600r through 1,500r exposures were collected until death. At an 1,800r exposure the organism enclosed as an adult but oviposited for only 3 days after which it was sterile until day 8 when death occurred. Only one adult developed from the 2,100r exposure. She failed to oviposit and died on day 3. On the basis of fecundity as well as survival to adulthood, a 2,100r exposure appeared definitely damaging.

On the basis of adult longevity, wasps irradiated as embryos were damaged by a 900r exposure with a striking effect at a

[1] An observation from research leading to partial fulfillment of the requirements for the Doctor of Philosophy degree at North Carolina State College, Raleigh, N. C. This paper was communicated and endorsed for publication by Jared J. Davis, Richland, Washington. Accepted for publication April 19, 1960.

[2] Appreciation is expressed to Dr. A. M. Clark of the University of Delaware for drawing this feature of data to my attention. It is my understanding that Dr. Clark has obtained considerable substantiating evidence on the matter. (Personal communication).

Table 1.—Performance of adult females x-rayed as 24-hour-old embryos.

X-RAY IN r	ADULT FEMALES ECLOSED (%)	IRRADIATED MOTHER'S	
		Egg Hatchability (%)	Longevity (days)
600	18.5	82.9	23+
900	25.9	82.7	16
1,200	16.7	85.1	14
1,500	11.6	84.3	8
1,800	17.6	81.3	8
2,100	2.1	No eggs	
2,400	0.0		

1,500r dose (table 1). Thus, adult longevity compared with induction of sterility or immediate lethality, appears to be the most sensitive criterion of irradiation damage at this developmental stage. The mechanism for life shortening by ionizing irradiations without serious effects upon reproductive tissues should be more completely studied.

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Malathion Residues in the Tissues of Sheep, Goats, and Hogs[1]

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Malathion has been shown by several investigators to be effective at 0.5% against lice on cattle, goats, and hogs and against sarcoptic mange of hogs (Smith & Richards 1955, Raun 1956, Johnson 1958). Toxicity studies have shown that a dermal application of 0.5% to baby calves and 2% to adult cattle does not produce visible symptoms of poisoning (Radeleff & Woodard 1957). The treatments made by Raun did not produce gross symptoms of toxicity or cause skin irritation in hogs when applied at concentrations as high as 2%.

These studies indicated that malathion could be safely applied to animals at effective levels, but before it could be recommended for louse control it was necessary to determine the residues in the tissues. The storage of malathion in the fat of beef

Table 1.—Malathion residues (p.p.m.) in the tissues of sheep and goats 1 week after two treatments with 1% malathion emulsion.

ANIMAL	LEAN MEAT	OMENTAL FAT	RENAL FAT
Sheep:			
In fleece	0.10	0.07	0
Shorn	.08[a]	.41	0
Control	.15	.28	0.72
Goats:			
In fleece	.02[a]	3.20	0
Shorn	.05[a]	—	—
Control	.15	.20	0.45

[a] Investigators in the American Cyanamid Company found 0.14 p.p.m. in the shorn sheep and goat and 0.03 p.p.m. in the goat in fleece, but no residues in any of the other tissues, including those of liver.

cattle and its secretion in the milk of dairy cows have been reported by Claborn *et al.* (1956). To widen the scope of application, experiments were conducted to determine malathion residues in sheep, goats, and hogs following spray applications of twice the effective concentrations.

Two hogs, three sheep, and three goats were sprayed twice, a week apart, with 1% malathion. One of the hogs was treated with an emulsion and one with a suspension. The goats and sheep were sprayed with an emulsion. One goat and one sheep were not treated, and in each group of the treated animals, one was shorn and one was in full fleece.

One week after the second treatment the animals were slaughtered. Samples of liver, lean meat, and omental and renal fat were collected from the goats and sheep, and liver, renal fat, and ham from the hogs. The malathion in hog samples was determined by the colorimetric method described by Norris *et al.* (1954) and that in the samples from the sheep and goats by a similar method described later (Norris *et al.* 1958).

The results of analyses of the sheep and goat tissues are presented in table 1. No residues were found in the tissues of either of the hogs,[5] and very little was found in the tissues of any of the treated animals, except in the goat omental fat. This figure could not be rechecked because of the small size of the sample.

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[2] Entomology Research Division.
[3] Animal Disease and Parasite Research Division.
[4] In cooperation with the American Cyanamid Company.
[5] Data furnished by the American Cyanamid Company.

These results indicate that any malathion that is absorbed is rapidly excreted within a week. The lack of residues from the 1% sprays suggests that there is even less hazard from the 0.5% concentration.

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Effects of Low Temperatures on the Eggs of the Angoumois Grain Moth,[1] the Indian-Meal Moth,[2] and the Confused Flour Beetle[3, 4]

VICTOR E. ADLER[5]

The tests reported in this paper were conducted to determine the effects of low temperatures on the eggs of the Angoumois

[1] *Sitotroga cerealella* (Oliv.)

[2] *Plodia interpunctella* (Hbn.)

[3] *Tribolium confusum* Duv.

[4] This report is a part of a thesis presented to the Graduate School of Kansas State University in partial fulfillment of the requirement for the Master of Science Degree. Accepted for publication June 13, 1960.

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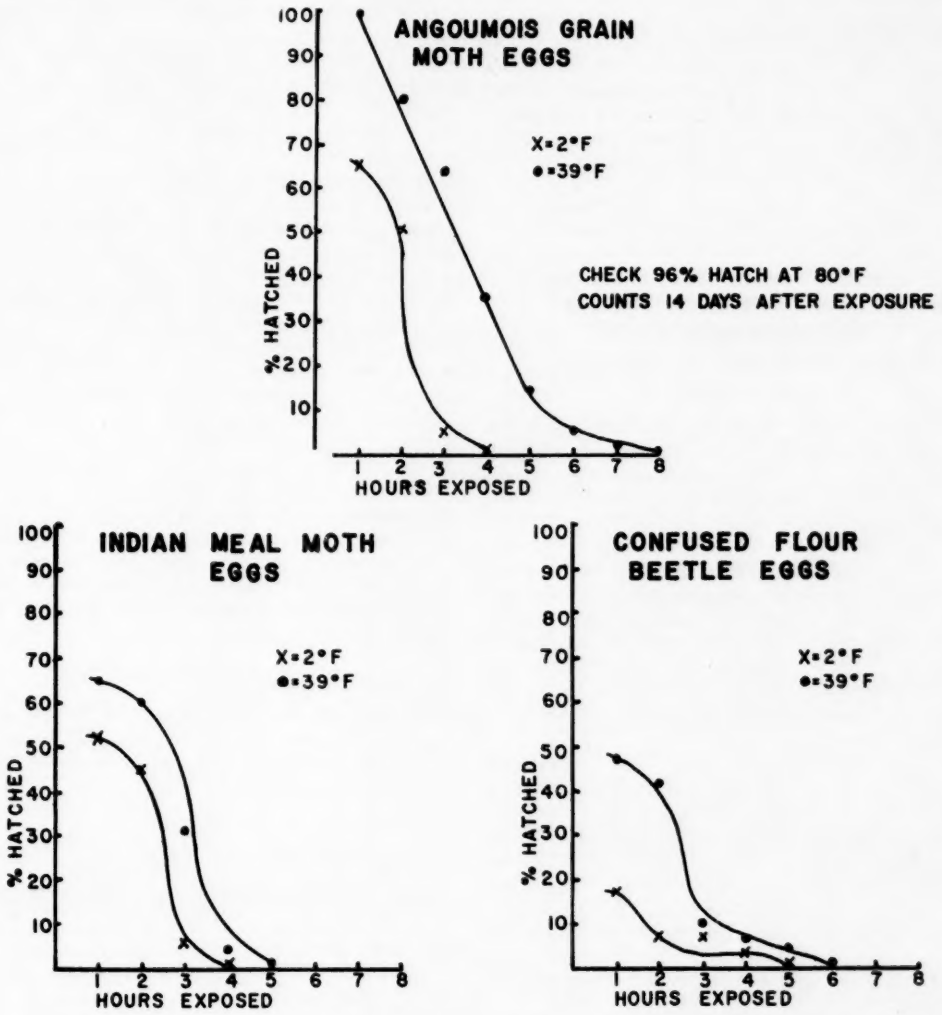

FIG. 1.—Percentages of Angoumois grain moth, Indian-meal moth, and confused flour beetle eggs hatching after exposure for different lengths of time to temperatures of 2° F. and 39° F.

grain moth, the Indian-meal moth, and the confused flour beetle. A 12-cubic foot deep freeze and a 6-cubic foot refrigerator were used in this experiment to provide temperatures of 2° F. and 39° F.

Angoumois grain moth eggs were subjected to both temperatures for periods of from 1 to 8 hours, and incubated for 14 days. The number of hatched eggs was then recorded and the percentages computed.

No eggs were observed to hatch after an exposure of 4 hours at 2° F., whereas a 1-hour exposure at that temperature resulted in a 65% hatch; at 39°, an 8-hour exposure inhibited all hatching, whereas a 1-hour exposure at this temperature yielded a 100% hatch (fig. 1). Ninety-six per cent of the control eggs hatched. From these data, it was determined that the LE_{50} (lethal exposure to effect a 50% hatch) is 2 hours at 2° F. and 3.3 hours at 39° (fig. 1).

Comparable studies were made on Indian-meal moth eggs. The eggs were exposed from 1 to 5 hours at 2° F. and 39° F. After a 4-hour exposure at 2°, no hatching was observed; a 1-hour exposure at that temperature resulted in a 52% hatch; at 39°, a 5-hour exposure totally inhibited hatching but a 1-hour exposure yielded a 65% hatch (fig. 1). Thus, the LE_{50} at 2° is 1.6 hours and at 39° is 2.5 hours. Ninety-six per cent of the untreated eggs hatched.

Confused flour beetle eggs were studied in a similar manner. These eggs were exposed 1 to 6 hours at 2° F. and 39° F. At 2° an exposure of 5 hours inhibited hatching of all eggs but a 1-hour exposure resulted in a 17% hatch; at 39° a 6-hour exposure totally inhibited all hatching whereas a 1-hour exposure yielded a 47% hatch (fig. 1). Thus, the LE_{50} at 2° is much less than 1 hour and at 39° it is 0.9 of an hour. Ninety-six per cent of the control eggs hatched.

The Effectiveness of Sevin-Sesamex Combinations Against Adult Flour Beetles (*Tribolium* spp.)[1]

ROY D. SPEIRS[2], *Stored-Product Insects Laboratory, Savannah, Georgia*[3]

Sevin® (1-naphthyl methylcarbamate) was included in the preliminary evaluation program conducted by the Stored-Product Insects Laboratory, Agricultural Marketing Service, Savannah, Georgia. Preliminary evaluations of Sevin by Speirs and Oglesby in 1958 (unpublished report) showed that relatively high rates of application of Sevin, compared with malathion, were required to produce high mortalities to flour beetle adults consisting of a mixed culture of *Tribolium confusum* Duv. and *T. castaneum* (Hbst.) and black carpet beetle larvae, *Attagenus piceus* (Oliv.). Eldefrawi *et al.* (1959) found that sesamex acted as a synergist for Sevin when applied to house flies. In the tests described in this paper, selected combinations of Sevin and sesamex applied at various rates were evaluated for their direct contact toxicity to flour beetle adults.

MATERIALS AND METHODS.—The adult flour beetles were reared in a controlled condition room maintained at a temperature of 80° ± 2° F. and a relative humidity of 60 ± 5%, and were from 1 to 30 days of age. The rearing medium consisted of white flour and corn meal containing 5% of brewer's yeast. Sixteen petri dishes of 10 insects each were treated at each rate of application. A total of 1,920 beetles was used. The insects were exposed for one-half hour to settling aerosols while retained in open, paper-lined petri dish halves on the paper-covered floors of four closed, cylindrical, stainless steel towers, 6 feet high and 2 feet in diameter.

Immediately after exposure the insects were transferred to clean petri dishes with paper-lined bottoms and stored at the same temperature and humidity shown above for observations of the number of inactive (I) and dead and moribund (D+M) insects at various time intervals.

Table 1.—Direct contact toxicity of Sevin, sesamex, and combinations of Sevin and sesamex to flour beetle adults.

Insecticides and Ratio	Intended Deposit of Insecticides (mg./sq. ft.)	Post Exposure (hours) 24 I[1] (%)	24 D+M[2] (%)	120 I[1] (%)	120 D+M[2] (%)
Sevin+sesamex					
1:5	10.4+52.0	100	97	100	100
1:5	3.5+17.5	87	78	84	83
1:3	3.5+10.4	88	80	88	88
1:1	3.5+ 3.5	76	73	76	76
2:1	3.5+ 1.8	63	49	62	62
3:1	3.5+ 1.2	46	40	44	42
5:1	3.5+ 0.7	53	46	55	55
1:5	0.9+ 4.4	23	20	22	22
Sevin	10.4	88	76	78	78
	3.5	26	23	29	29
Sesamex	52.0	0	0	0	0
Check (solvent)	—	0	0	0	0

[1] Inactive.
[2] Dead plus moribund.

The technical grade Sevin, sesamex, and combinations of Sevin and sesamex were formulated in a solvent composed of 50:50 (w/w) mixture of deodorized kerosene and tetrachloroethylene mixed with ethanol in equal parts by volume. The formulations were applied through the center of the tower top by introducing 2 milliliters from a pipette through an air atomizing nozzle with an air pressure of 10 pounds per square inch. The concentration of ingredients in the formulations was adjusted to give the calculated rates of application shown in table 1 (based on a fallout rate of 80% of introduced formulation). Treatments were applied at a temperature of 75° to 80° F.

RESULTS AND DISCUSSION.—The formulations combining Sevin and sesamex produced higher mortalities to flour beetle adults than were produced by formulations of Sevin alone applied at equivalent rates of application (table 1). Equal mortalities were obtained with Sevin applied at 10.4 mg./sq. ft. and with a combination treatment of Sevin and sesamex (1:1) applied at 3.5 to 3.5 mg./sq. ft., even though the combination deposited about two-thirds less Sevin.

The increased effectiveness of Sevin-sesamex combinations over Sevin alone against *Tribolium* adults was not as great as that obtained by Eldefrawi *et al.* (1959) with house flies. However, the direct contact toxicity of the Sevin-sesamex combinations shown in these tests makes Sevin a promising candidate insecticide which should undergo additional evaluation tests to determine fully its potential in the control of stored-product insects.

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[1] Accepted for publication June 27, 1960.
[2] Joe H. Lang assisted in these laboratory tests.
[3] This is one of the field stations of the Stored-Product Insects Branch, Market Quality Research Division, Agricultural Marketing Service, U. S. Department of Agriculture.

Responses of Raisin Moth Larvae to Residues of Six Insecticidal Sprays[1]

PEREZ SIMMONS,[2] *U. S. Department of Agriculture, Agricultural Marketing Service, Market Quality Research Division, Fresno, California*

When fully grown, larvae of the raisin moth, *Ephestia figulilella* Greg., leave the food where they developed and seek dark, dry, tight places in which to spin cocoons for pupation. Fumigation takes care of most such larvae, but some appear in raisin storage yards in raisins recently received and not yet covered with laminated paper and fumigated. In their wanderings a large percentage of these larvae crawl around on the stacks of boxes of unprocessed raisins that are covered with laminated paper. The purpose of the laboratory tests described in this paper was to find insecticidal sprays that would kill larvae of the raisin moth when applied to the paper coverings.

During December 1954 and January and February 1955 a 25% malathion wettable powder was used to make the spray for the first tests. Pieces of heavy sisalkraft paper, 1 by 2 feet, were sprayed until run-off occurred. The formulation contained the equivalent of 8.3 pounds of malathion in 100 gallons of water and was applied at the rate of 2.3 gallons per 1,000 square feet or a calculated deposit of 86 milligrams of malathion per square foot. The sprayed papers were dried for 24 hours before the first exposure was made.

There were 550 exposures (5 larvae for each), none of which were replicated. The larvae were kept on the treated surfaces by means of a brush or by rotating the papers with reference to the light source since the larvae are negatively phototropic. After exposure the larvae were transferred to and held in petri dishes.

Five larvae were placed on the treated papers for each of five exposure periods: 15, 30, 45, 60, and 120 minutes. Additional exposures were made for the same lengths of time every 24 hours up to 11 days. Deposits tested were 1 to 11 days old. Mortalities were recorded daily until all the larvae died or for 10 days if death did not occur sooner.

Larvae exposed on the malathion deposits were paralyzed one to several days before they died. An immobile paralyzed larva is as ineffective as a dead one, and for this reason dead and paralyzed insects in this series were reacorded as dead. A separate record was made of the day all larvae were found dead. Even those that survived were less active than larvae in the check lots. Survivors were not held for observation on the condition of adults. Some of the data on complete mortalities of larvae exposed on deposits of different ages, excluding paralyzed larvae, are tabulated below.

Minutes of Exposure	*Age of Deposits (days)*	*Days for Complete Mortality*
15	3	6
	4	8
	5	6
30	4	6
	5	5
	7	6
45	3	4
60	3	4
120	3	4

Deposits 10 or 11 days old showed marked loss of killing power, regardless of exposure time.

The 1-day-old deposits never killed or paralyzed the insects as quickly as the deposits a few days older. There was almost no mortality among check insects.

Another series of exposures was made in 1955 and 1956, using the same procedures but varying the spray formulations. A few tests with 50% DDT wettable powder spray were included in this series, but the formulation used (8 pounds in 100 gallons of water, or a calculated deposit of 83 milligrams per square foot) was ineffective.

Malathion wettable powder, 25% concentration, was tested at strengths equivalent to 10, 5, and 4 pounds of actual insecticide in 100 gallons of water. The respective calculated deposits were 104, 52, and 41 milligrams per square foot. Five larvae were exposed on each age of deposit. As a rule this was done twice, but some exposures were not repeated and others were done three times. As in the earlier work exposures were for 15, 30, 45, 60, and 120 minutes. Age of deposit controlled rapidity of death more than did length of exposure. Deposits 2, 3, or 4 days old were more rapid in their action than those that were 1 day old. The 10-pound dosage rate usually killed in 5 or 6 days if the deposits were 3 to 9 days old, but when the deposits were 10 and 11 days old their insecticidal effect became weakened.

Four- and five-pound concentrations of malathion were relatively ineffective. The 5-pound strength gave the earliest 100% mortality 2 days after a 60-minute exposure to a deposit 7 days old. As in previous work, 1-day-old deposits proved to be less potent than those several days older.

In general, results with the 4-pound strength resembled those with the 5-pound concentration. Complete mortality occurred earliest after a 45-minute exposure on a deposit 6 days old. This was recorded on the third day after exposure. Four-pound deposits 9 days old performed poorly.

Usually all check larvae survived through the observation periods of 10 to 14 days.

In 1955, 1956, and 1957 exposures of raisin moth larvae, usually in lots of five and repeated once, were made on deposits from liquid sprays made by four formulations: a chlordane-lindane mixture, pyrethrum-piperonyl butoxide, chlordane alone, and lindane alone. The first mixture contained emulsifiable chlordane (72% technical) and emulsifiable lindane (13% gamma isomer of BHC). Four milliliters of the former and 1 milliliter of the latter were emulsified in 400 milliliters of water. Another formulation, of double strength, was tested also. Application to the 1-foot by 2-foot laminated papers was at the rate of 10 milliliters per square foot, equivalent to 2.6 gallons per 1,000 square feet. Use of the papers coated with the first mixture began after they had dried for at least 1 day. Exposures on double-strength deposits began the day the papers were sprayed. Periods of exposure were 15, 30, 45, 60, and 120 minutes. Results were poor; no mortalities above 70% were recorded during 10 days of observation.

A concentrate containing 1% pyrethrins, 10% piperonyl butoxide, and 72% mineral spirits was diluted at the rate of 50 milliliters in 450 milliliters of water. This formulation was sprayed on the paper sheets at the rate of 11 milliliters per square foot. Exposures were made when the dried deposits were 0, 1, and 2 days old. Irregular observations were made for 13 or 14 days. The value of the records was impaired by the occurrence of disease among the treated larvae. Check lots of larvae were not affected. Kills of exposed larvae were poor.

Two formulations containing chlordane were tested. The first was made by adding 100% chlordane to acetone at the rate of 0.9595 gram per 100 milliliters or 8 pounds per 100 gallons. Application was 11 milliliters per square foot. Larvae were in contact with deposits 2 days and 5 days old for 15, 30, 45, 60, and 120 minutes. Results were strongly negative.

A wettable powder containing 40% technical chlordane[3] was used in the second series of exposures. Twelve grams in 500 milliliters of water gave a concentration of 8 pounds chlordane per 100 gallons. Film ages when raisin moth larvae were released

[1] Accepted for publication June 30, 1960.

[2] The tests reported in this note were conducted by Dwight F. Barnes (deceased), Joseph P. Bernhard (resigned February 1955), Erwin F. Sawall, Jr. (resigned January 1956), and Samuel B. Stagg (resigned June 1958).

[3] Equivalent to 24% Octachloro-4, 7-methano-tetrahydroindane and 16% related compounds. Inert ingredients (clay filler, wetting agent) 60%.

on the treated paper sheets were 1, 2, 3, and 4 days. Each length of exposure was tested with 10 larvae in 2 runs of 5 each. These exposures gave unsatisfactory results.

Acetone was the carrier for 100% lindane. The insecticide was added to acetone at the rate of 1 pound per 100 gallons and the experimental batches were made by using 1.119 grams in 100 milliliters. Twenty-five milliliters were sprayed on 2 square feet of stack paper. Deposits 1 day old were the only ones tested. Contacts lasted 15, 30, 45, 60, and 120 minutes. These exposures gave poor kills.

DISCUSSION.—Deposits of malathion applied to test papers at concentrations of 8 or 10 pounds of malathion in 100 gallons of water gave many complete kills of raisin moth larvae 3, 4, or 5 days after exposure to coatings 2, 3, or 4 days old. Fresh (1-day-old) deposits were less effective than those that were somewhat older, and deposits 11 days old had lost most of their potency. DDT, chlordane-lindane mixture, pyrethrum-piperonyl butoxide, chlordane alone, and lindane alone performed poorly at the strengths, ages, and exposure periods tested.

Table 1.—Effect of insecticidal sprays on hornworms and on parasitism of hornworms by *Apanteles congregatus*.

MATERIAL[a]	ACTUAL PER ACRE	RESIDUES (P.P.M.)	HORNWORMS PER PLANT[b]			HEALTHY PER PARASITISED HORNWORM
			No. Healthy	Control (%)	No. Parasitised	
Endrin	0.2 lb.	1.4	1.43	50	0.10	14.0
	0.4 lb.	1.5	1.17	59	.21	5.5
	0.8 lb.	4.3	.14	95	0	∞
TDE	1 lb.	27	.50	82	.03	14.0
	2 lb.	82	.49	83	.02	25.0
	4 lb.	—	.28	90	.08	3.5
Blank	4 pt.	—	2.52		1.35	1.9
	16 pt.	—	2.40		.88	2.7
Check	—	—	2.85		1.25	2.3
Insecticide			.64		.075	8.5
Noninsecticide			2.57		1.155	2.2

[a] Materials applied on August 11, 1959.
[b] Counts made on September 16, 1959.

Effect of Insecticidal Sprays on Hornworms and on *Apanteles congregatus*[1]

RICHARD THURSTON, *Kentucky Agricultural Experiment Station, Lexington*

TDE and endrin are the two most commonly used insecticides on field tobacco in Kentucky. Both of these are long residual chemicals and might be expected to give control of insects over long periods of time.

In 1959, experimental plots of burley tobacco, designed primarily for tests on the phytotoxicity of solvents, were sprayed with various dosages of endrin and TDE emulsifiable concentrates and with emulsifiable concentrates which contained no insecticide (table 1). Endrin emulsifiable concentrates were 1.6 lb./gal and TDE emulsifiable concentrates were 2 lb./gal. formulations. Blanks (emulsifiable concentrates with no insecticide) were applied at levels equal to the amount of emulsifiable concentrate used for the high endrin and the high TDE dosages. The plots were 30 feet long and 3 rows (10′) wide with four replicates per treatment. The first 10 feet of each treated plot was sprayed on June 30, the first 20 feet on July 21, and the whole 30 feet on August 11. The sprays were applied with compressed-air, hand sprayers with fan-type nozzles. Ten gallons of spray per acre were used. The single nozzle was passed once directly over the top of the plants at a distance of 12 to 15 inches from the bud.

On September 16, approximately 5 weeks after the last spraying, counts were made of the healthy and the parasitised hornworms in the middle row of each plot. More than 95% of the hornworms were *Protoparce sexta* (Johan.) and less than 5% were *Protoparce quinquemaculata* (Haw.). On September 17, samples of leaves from plants in each of the sub-plots were harvested, frozen, and shipped to the Pesticide Residue Laboratory at North Carolina State College[2] for residue analyses. No samples for residue analysis were taken of the 4-pound-per-acre rate of TDE.

Results of the insect counts and of the residue analyses are given in table 1. Endrin at 0.2 and 0.4 pound per acre was not so effective as any of the TDE treatments, but at 0.8 pound per acre it was as effective as the TDE treatments. TDE at all three dosages gave good control of hornworms. The residues, which are averages of the analyses of the three 10-foot sub-plots of each treatment, show that TDE left considerably larger residues per unit applied than endrin. The recommended dosage of TDE, 1 pound per acre, left approximately 20 times more residue than the recommended dosage of endrin, 0.2 pound per acre. The 0.4-pound-per-acre endrin treatment left residues only slightly higher than the 0.2-pound endrin treatment. This may explain the relatively small difference in control obtained from these two treatments.

Parasitism of the hornworms by *Apanteles congregatus* (Say) was greatly affected by the insecticide treatments. The insecticide-treated plots, when grouped together, showed only 10% parasitism but the noninsecticide plots, checks and blanks grouped together, averaged 31% parasitism. At least three explanations may be postulated for this reduction in parasitism in the insecticide-treated plots. (1) The number of hornworms in the insecticide-treated plots may have been low enough to make it difficult for the adult parasites to find their hosts in these plots. (2) The amount of TDE or endrin on the leaves ingested by the hornworms may have been too low to kill some of the hornworms but high enough to kill the larvae of the tiny wasplike insect developing inside the hornworm. (3) The residues on the tobacco plant may not have been high enough to kill the hornworms but high enough to kill the adult parasites. The first explanation seems doubtful because even the insecticide-treated plots with large numbers of hornworms, the 0.2- and 0.4-pound-per-acre endrin plots, had low levels of parasitism. Populations averaging 1.43 and 1.17 hornworms per plant would appear to be high enough to make hosts as readily available as populations even twice that high. Also, in insecticide-treated plots, where toxicity to the parasites could be eliminated, the ratio of healthy to parasitised hornworms would be expected to be lower than in untreated plots; repeated observations indicate that parasitised hornworms are much more difficult to kill than nonparasitised hornworms. No work was done to determine if ingestion of nonlethal dosages of TDE or endrin by the hornworm would kill larvae of *Apanteles congregatus*. However, whatever the explanation, it appears that these two insecticides are responsible for significant reductions in populations of this parasite.

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published with approval of the Director. Partial cost of publication of this paper was met by the Kentucky Agricultural Experiment Station. Accepted for publication June 20, 1960.
[2] The author wishes to thank Dr. Thomas Bowery of North Carolina State College, Raleigh, North Carolina, for conducting the residue analyses.

BOOK REVIEW

DISEASES AND PESTS OF ORNAMENTAL PLANTS, by PASCAL P. PIRONE, BERNARD O. DODGE, and HAROLD W. RICKETT, New York Botanical Garden. 6″×9″, i–x+775 pp., 221 figs. ed. 3. The Ronald Press Company, New York 10, N. Y. $10.00.

The standard reference book has been thoroughly reworked by Pirone. It is larger, has more illustrations, and discusses modern control measures. Part I deals with the various kinds of diseases and pests; the chapter on control includes uses, rates, and hazards of some newer pesticides. Part II, Diseases and Pests of Particular Hosts, is arranged according to scientific names of some 500 host plants, but common names are indexed. Generic names heading each page are helpful in finding the pests afflicting a particular plant.

Technical workers will miss references to literature, but these are so numerous that they could scarcely be included. The home gardener will find difficulty in identifying various pests with sufficient accuracy to select the proper control measures. Chemicals for pest control not available to the home gardener are included, and some of the pesticides have not been registered for the indicated use; *e.g.*, phorate (Thimet) for control of iris borer, endrin and schradan for cyclamen mite on African violets, and parathion for various pests. The home gardener would find pesticides easier to prepare if rates were given in teaspoons to make one or more gallons of spray.

Some errors were noted in this third edition. On page 32 the statement that most virus diseases cause death of infected plants is too strong. Figure 114, reproduced from Edition 1, as physiological chlorosis appears to be camellia yellow spot, a virus disease. Figure 133, also reproduced from Edition 1, again ascribes Forsythia galls to *Phomopsis*. Although N. H. Brown reported *Phomopsis* galls on *Jasminum*, *Ligustrum*, and *Viburnum*, there seems to be no published proof that this fungus causes the galls on Forsythia.

Both the lesser bulb fly, *Eumerus tuberculatus* Rond., and the narcissus bulb fly, *Lampetia equestris* (F.), are treated as primary pests of narcissus and amaryllis. Injury to amaryllis (Figure 106) is typical of *Lampetia*, not of *Eumerus*. The obligate feeding of the boxelder bug on seeds of pistillate boxelder trees and the consequent control by growing only staminate trees are not mentioned. The characteristic damage to delphinium caused by *Aphis rociadae* Ckll. is attributed (p. 319) to *Macrosiphum rudbeckiae* Fitch.

The flower thrips (*Frankliniella tritici* Fitch) not the greenhouse thrips, is responsible for injury to peony flowers. Conversely, the hemerocallis thrips (*Frankliniella hemerocallis* Crawford), not the flower thrips, causes the bronzing damage to stems and buds of hemerocallis. The so-called privet weevil, *Pseudocneorrhinus setosus* Roelofs (p. 462) is properly referred to as the Japanese weevil, *Pseudocneorhinus bifasciatus*. Privet in the South is infested by the citrus whitefly, not the greenhouse whitefly (p. 462). The marked crinkling of young privet leaves alluded to as aphid injury is doubtless feeding injury of Eriophyid mites.

Most types of pesticide application equipment, but not the increasingly popular hose sprayers, the power-driven knapsack duster-sprayers, and the push button spray cans, are mentioned.

The illustrations are excellent. In spite of the errors noted the book is a valuable contribution and should be available to all research and extension people whose work is related to pests of ornamentals. Diseases and pests of 500 genera of plants cover a very broad field for a single book. Some omissions are to be expected and some errors are pardonable.—

PHILIP BRIERLEY AND FLOYD F. SMITH,
U. S. Department of Agriculture

INDEX XVI

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